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Continuum and discrete approach in modeling biofilm development and structure: a review

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Abstract The scientific community has recognized that almost 99% of the microbial life on earth is represented by biofilms. Considering the impacts of their sessile lifestyle on both natural and human activities, extensive experimental activity has been carried out to understand how biofilms grow and interact with the environment. Many mathematical models have also been developed to simulate and elucidate the main processes characterizing the biofilm growth. Two main mathematical approaches for biomass representation can be distinguished: *continuum* and *discrete*. This review is aimed at

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exploring the main characteristics of each approach. Continuum models can simulate the biofilm processes in a quantitative and deterministic way. However, they require a multidimensional formulation to take into account the biofilm spatial heterogeneity, which makes the models quite complicated, requiring significant computational effort. Discrete models are more recent and can represent the typical multidimensional structural heterogeneity of biofilm reflecting the experimental expectations, but they generate computational results including elements of randomness and introduce stochastic effects into the solutions.

Keywords Biofilm · Biomass representation · Continuum models · Discrete models

Mathematics Subject Classification 35K57 · 35K65 · 35L50 · 37B15 · 68Q80 · 76D05 · 76T30 · 76Z05 · 91B70 · 9202 · 9208 · 92B05 · 92D25

1 Introduction

Recent advances in quantitative recovery and in direct observation of microbial populations have revealed that biofilms represent the prevailing structures in microbial lifestyle (Costerton et al. 1995; Flemming 2014). In most natural and human environments, biofilms are constituted by highly structured multispecies communities composed of millions of microorganisms that accumulate on surfaces and secrete extracellular polymeric substances (EPS), which anchor the cells to each other as well as to the surfaces (Costerton 1995; Davey and O'toole 2000; Jenkinson and Lappin-Scott 2001; Klapper and Dockery 2002, 2010; Stoodley et al. 2002; Tolker-Nielsen and Molin 2000; Van Loosdrecht et al. 1995; Watnick and Kolter 2000). The secreted adhesive matrix represents a primary component of the biofilm as usually constitutes 50 to 90% of the total dry mass (Flemming and Wingender 2010) and affects the internal structural organization of the biofilm, mainly in terms of mechanical properties (Billings et al. 2015).

The bacteria living in a biofilm are not randomly distributed but they live in distinct niches and they benefit from interspecies cooperation (Elias and Banin 2012) showing more resistance to toxic substances such as antibiotics, chlorine and detergents thanks to diffusion barriers (Costerton et al. 1994). Biofilms are important components of food chains and are involved in self-purification processes in soil, water and sediments and in the biodegradation of organic compounds including the environmental pollutants. They have been used to treat wastewater since the end of the nineteenth century (Nicolella et al. 2000). Compared to suspended cells, the bacteria growing in biofilms show some advantages: (i) they cannot be washed away with the water flow; (ii) they show an increased resistance to antimicrobial agents and allow the achievement of a higher biomass concentration value in bioreactors; (iii) their heterogeneous physical structure deriving from the interplay of diffusion and consumption of nutrients leads to the formation of microniches where several bacterial species coexist and contribute to the treatment of different organic and inorganic substrates (Wang and Zhang 2010). At the same time, biofilms can have a significant impact on the surrounding environment, including biofouling, biocorrosion, oil field souring and infections in host tissues or medical implants (Klapper and Dockery 2002).

Biofilm formation is a dynamic process resulting from the balance of several physical (substrate transport, detachment, etc.) and biochemical factors (microbial growth, substrate conversion, etc.) (D'Acunto and Frunzo 2011; Monds and O'Toole 2009; Toyofuku et al. 2016). The formation of various biofilm architectures and the related activities are strongly affected by the specific environmental conditions, such as electron donors and acceptors levels, hydrodynamic conditions, carbon source, etc. (Paul et al. 2012), in which the sessile communities grow. For example, porous biofilms with channels and voids between the finger-like or mushroom outgrowths are typical of a substrate-transport-limited regime. Instead, compact and smooth biofilms occur when the biomass growth rate is limiting or the shear stress is high (Van Loosdrecht et al. 1995).

Mathematical modeling of biofilm expansion in flows has been widely performed during the last decades (for a survey see Wanner et al. 2006; Klapper and Dockery 2010; Wang and Zhang 2010; Horn and Lackner 2014). Biofilm models represent a perfect means to understand the basic principles determining biofilm formation, composition, structure and function (Noguera et al. 1999a) and therefore they can be used to effectively utilize and control biofilms in industrial and medical settings (Picioreanu et al. 2004b). Mathematical models come in many forms that can range from very simple empirical correlations to sophisticated and computationally intensive algorithms that describe three-dimensional (3D) biofilm morphology and activity (Wanner et al. 2006). The domain of interest is usually divided in three compartments: the bulk liquid, the boundary layer and the biofilm itself (Fig. 1).

All biofilm models simulate the dynamics of two types of components, particulate (active and inert biomass, EPS) and dissolved (substrates and metabolic products), and generally include three main elements: transport mechanism; consumption and growth mechanism; and loss mechanism (Klapper and Dockery 2002). The transport of dissolved compounds within the biofilm matrix is governed by diffusion. It plays a crucial role in biofilm development, since the concentrations of nutrients and products determine the rates of microbial reactions as well as all the processes that generate an increase in volume are driven by nutrient availability (Picioreanu et al. 2000a). Moreover, substrate concentration gradients within the biofilm contribute to the formation of different environmental niches (Stewart 2003). Biomass growth kinetics depends on substrate concentrations; by consuming substrate, bacteria grow and duplicate, they produce exopolymeric substances determining an increase in the biofilm volume, usually called biomass spreading. The biofilm models have proposed different approaches (consisting of stochastic individual based models, stochastic cellular automata models and a variety of deterministic partial differential equation models (Emerenini et al. 2015)) to describe the spreading of the newly formed amount of biomass, each of them characterized by its own strength and weaknesses as it will be highlighted in the next sections. For biofilms growing in hydrated environments, loss mechanisms are strictly related to the external fluid flow which can cause biofilm deformation, breakup and detachment (Tierra et al. 2015). The latter is a determining factor for biofilm-structure formation (Derlon et al. 2013; Morgenroth 2003). It represents the primary process that balances microbial growth and, thereby, determines the steady state accumulation of the biofilm and the overall biofilm activity (Picioreanu et al. 2000a; Stewart 1993),

and it greatly affects the performance and the stability of biofilm reactors (Picioreanu et al. 2001).

However, the biofilm concept has drastically evolved over the years: the recent experimental activities have revealed a rich diversity in biofilm structure, functions and properties and have led to the formulation of mathematical models aimed at reflecting this biological, ecological and physical complexity (Cogan et al. 2011). In this context, extensive research activity has been devoted during the last decade towards the mathematical modeling of biofilm mechanical/physical properties and interactions with the surrounding fluid flow, in terms of deformation and detachment. Up to date, it represents one of the main sources of shortcomings in this research field (Cogan et al. 2011). Due to the economic and human impacts of biofilm-based infections, consistent effort has been devoted to the mathematical modeling of biofilm tolerance mechanisms (the most accredited hypothesis refers to a physiological protection Cogan 2008), in order to outline the treatment and eradication of biofilms (see Cogan 2013, for a survey up to 2013). Moreover, specific ecological and biological aspects have been progressively taken into account: among them the mathematical modeling of Quorum Sensing (QS) and persistence has gained an increased attention in the context of biofilms.

The review presented herein focuses on the description of the different modeling approaches used to treat the biomass and simulate the biomass transport mechanism. Biofilm displacement is mainly caused by cell growth and division and EPS production, and can be affected by other processes changing the biofilm volume, such as attachment and detachment (Wanner et al. 2006). Over the last decades, biofilm models have reached a high level of complexity and have progressively incorporated a huge number of physico-chemical and biological processes. However, they can be classified in two broad categories based on biomass representation (Böl et al. 2013):

- *continuum models*, which do not take into account the behavior of an individual microorganism directly as they treat biomass as a unicuum, based on population-averaged behavior of different functional groups;
- *discrete models*, which are generally defined bottom-up models, since biofilm structure is not provided as an input to the model, but the complex morphology of biofilms emerges as a result of the actions and interactions of the biomass units with each other and the environment.

Each category is further subdivided by considering model dimensionality and the way in which diffusion and biomass spreading is treated. In particular, continuum models are classified in one-dimensional (1D) and multidimensional models; discrete models are divided in Cellular Automaton models, hybrid differential-discrete Cellular Automaton models and Individual-based Models (Fig. 1). Continuum models treat the dynamics of biomass spreading by using differential equations, widely used in mechanics and transport phenomena. In discrete models, biomass spreading is assumed to be a stochastic process. Over the years, specific biofilm features have been incorporated in both continuum and discrete models. However, in this article we are not going to review all biofilm models and related applications as they can greatly differ in terms of mathematical concepts and purposes they were developed for (Rahman et al. 2015). Instead, this work is presenting some of the most relevant modeling

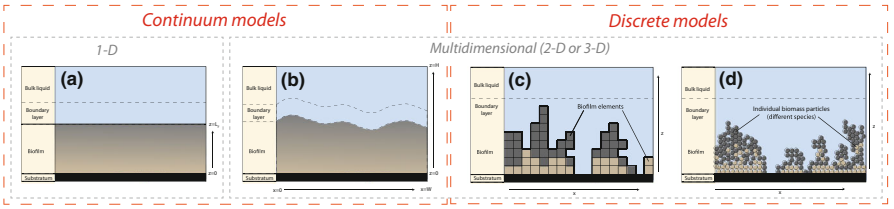

Fig. 1 Schematic representation of biofilm model classification adopted in this review and based on biomass representation and dimensionality: **a** 1D continuum models, **b** multidimensional continuum models, **c** Cellular automata, **d** individual based models (for multidimensional models only the 2D representation has been reported)

approaches proposed for biomass representation and biofilm expansion in flows, and it is aimed at evaluating the main features of the analyzed models in order to enable readers to select an appropriate modeling tool based on their own needs. The main purpose of this work is to provide the reader with an up-to-date general overview of the various mathematical approaches developed for biofilm modeling. The main features and drawbacks characterizing each modeling approach have been reported so as to avoid any filtering from the authors, which might imply their modeling orientation. Bearing that in mind, this review could be addressed to early-stage researchers who are moving the first steps in this research area or to scientists who already work in some areas of biofilm mathematical modeling and are interested in gaining insights on a different direction. In each section, the description of the models follows the chronological development they have undergone, starting from the pioneer works presented at the end of 70 s and moving to the works that have mainly contributed to the development and improvement of such research field. The paper is organized as follows. In Sect. 2, we introduce the continuum biofilm models as we consider both the system of partial differential equations characterizing biofilm development in one spatial dimension and the multidimensional formulation. Then, in Sect. 3, we deal with the discrete models which describe biofilm as generated by long-distance interactions with neighbors. In Sect. 4 we present some of the applications of the basic biofilm models reviewed in the previous sections. Section 5 contains some comments on the evaluation criteria of the continuum and discrete approach. Finally, Sect. 6 is a recapping of all the topics and an outline of the future research directions.

2 Continuum models

As the name implies, continuum models consider the domain of interest as a continuum (Bolea Albero et al. 2014) and biomass spreading as governed by differential equations. All continuum models are based on conservation laws which are formulated as balances of conserved properties (mass, volume, momentum, energy, etc.). For 1D models, these equations come in the form (Wanner and Reichert 1996)

$$\frac{\partial D}{\partial t} + \frac{\partial J}{\partial z} = R \tag{2.1}$$

where z is the space coordinate; t denotes the time variable; D represents a 1D property; J denotes the 1D property flux; R states the net property production rate.

Continuum models have undergone an evolution in terms of complexity: from 1D steady-state models, developed during the early 1970s, to multidimensional multispecies dynamical models that have been conceived during the last decade. This evolution has been influenced by the advances in computational and experimental tools and reflects the need of new biofilm models able to provide more complex two-dimensional (2D) or 3D descriptions of microbial biofilms, in agreement with experimental observations. Based on their dimensionality, continuum models have been classified in two groups: 1D continuum models and multidimensional continuum models. 1D models only consider the direction perpendicular to the substratum while multidimensional models neglect the concepts of uniform thickness and layering of biomass typical of 1D models, and allow the biofilm matrix to expand in more than one direction.

2.1 One dimensional continuum models-pioneer works

The first continuum biofilm models (Atkinson and Davies 1974; Williamson and McCarty 1976) have been developed in 1970 s in order to evaluate the substrate utilization kinetics in biofilms. These pioneer works were based on the concept that removing the substrates from an aqueous phase requires the diffusion of reactants into the biofilm, the metabolism by microorganisms and the diffusion of metabolic products through the biofilm and into the aqueous phase. These models can be considered the first example of continuum models since they were able to reproduce the essentials of biofilm development, idealizing the processes of substrate utilization, molecular diffusion and mass transport as simultaneous differential equations for a homogeneous layer of bacteria. In (Williamson and McCarty 1976), the authors adopted a schematic representation of the system where the biofilm is assumed to be attached to a flat surface with infinite length and width and characterized by a uniform cell density denoted X_f and a locally uniform thickness L_f. Substrate concentration within the biofilm changes only in the z direction, assumed perpendicular to the surface, and the rate of reaction is limited by a single substrate named rate-limiting substrate. The decrease in substrate concentration between the bulk liquid and the biofilm surface derives from an incomplete mixing of the liquid phase next to the biofilm surface coupled with mass transfer into biofilm and is modeled by the introduction of a liquid layer adjacent to and permeating the biofilm. In this layer the entire resistance to mass transport from the bulk liquid to the surface is concentrated. The depth of the diffusion layer L, is defined as the equivalent depth of liquid through which the actual turbulent mass transport can be described by molecular diffusion alone (Chaudhry and Beg 1998).

The model introduced in (Williamson and McCarty 1976) coupled the mass transport from the bulk liquid with the substrate biodegradation within the biofilm. In particular, the following elliptic equation was used to describe substrate utilization within the biofilm:

$$D_f \frac{\partial^2 S_j}{\partial z^2} = \frac{k X_f S_f}{K_S + S_f}, \quad 0 < z < L_f, \tag{2.2}$$

where D_f is the molecular diffusivity in biofilms $[L^2T^{-1}]$; S_f is the concentration of rate-limiting substrate at any point in biofilm $[ML^{-3}]$; X_f is the bacterial concentration within the biofilm, assumed constant with depth $[ML^{-3}]$; K_S is the Monod half velocity coefficient $[ML^{-3}]$; k is the maximum utilization rate of the rate-limiting substrate $[T^{-1}]$.

The rate of substrate utilization within the biofilm was modeled by a Monod-like bacterial kinetics and the diffusion flux through the diffusion layer and the biofilm by the Fick's law of diffusion. However, this pioneer work did not include any considerations on the growth and decay of the bacteria composing the biofilm.

The model proposed in (Williamson and McCarty 1976) was later improved by many researchers (Rittmann and McCarty 1980a, b, 1981; Rittman 1982; Rittmann and Dovantzis 1983; Rittmann and Brunner 1984) who amended the basic model of mass transport in a steady state biofilm with additional processes. Rittmann and McCarty (1980b) incorporated the expressions for biofilm growth and decay for a steady-state biofilm, which is defined as a biofilm that for a given bulk liquid substrate concentration has neither net growth nor decay. Later, Rittman (1982) introduced the biofilm loss rate caused by shear stress. This term was formulated as a first order expression similar to the term used for decay losses.

2.2 One dimensional continuum models

With the ongoing progress in experimental methods, more sophisticated multi-substrate-multispecies models have been developed (D'Acunto and Frunzo 2011, 2012; D'Acunto et al. 2011; Kissel et al. 1984; Lee and Park 2007; Rauch et al. 1999; Rittmann and Manem 1992; Rittmann et al. 2002; Tsuno et al. 2002; Wanner and Gujer 1984, 1986; Wanner and Reichert 1996; Reichert and Wanner 1997). These studies neglect the simplifying assumption of single-species biofilms and are mostly centered on the biofilm growth dynamics, including the biofilm thickness, the spatial distribution of microbial species and the substrate concentrations.

The 1D multispecies model of biofilm growth introduced by Wanner and Gujer (1984, 1986) has been successfully applied to many biofilm studies since its development and represents a pioneer work in the understanding of the complex bulk interactions characterizing multispecies biofilms. This model takes into account the following processes: (a) the simultaneous substrate utilization and diffusion within the biofilm; (b) the external mass-transport resistance from the bulk liquid to the biofilm surface; (c) the growth of new biomass proportional to substrate utilization; (d) the biomass loss from endogenous respiration and detachment, and (e) the formation of inert biomass. The following equations have been introduced (D'Acunto and Frunzo 2011; Wanner and Gujer 1986):

$$\frac{\partial X_i}{\partial t} + \frac{\partial}{\partial z}\left(uX_i\right) = \rho_i r_{M,i}\left(z, t, \mathbf{X}, \mathbf{S}\right), \quad i = 1, \ldots n, \quad 0 \le z \le L\left(t\right), \quad t > 0, \tag{2.3}$$

$$\frac{\partial u}{\partial z} = \sum_{i=1}^{n} r_{M,i} = G\left(z, t, \mathbf{X}, \mathbf{S}\right), \quad 0 < z \le L\left(t\right), \quad t > 0, \tag{2.4}$$

$$\dot{L}(t) = u(L(t), t) + \sigma_a(t) - \sigma_d(t), \quad t > 0, \tag{2.5}$$

$$\frac{\partial S_j}{\partial t} - \frac{\partial}{\partial z}\left(D_j \frac{\partial S_j}{\partial z}\right) = r_{S,j}(z, t, \mathbf{X}, \mathbf{S}), \quad 0 < z < L(t), \quad t > 0, \quad j = 1, \ldots, m, \tag{2.6}$$

where z is the biofilm growth direction assumed perpendicular to the substratum $[L]$; ρ_i denotes constant density $[ML^{-3}]$; $X_i(z,t) = \rho_i f_i$ denotes the concentration of microorganisms i, $\mathbf{X} = (X_1, \ldots, X_n)$ $[ML^{-3}]$; $f_i(z,t)$ is the volume fraction of microbial species i, $\sum_{i=1}^{n} f_i = 1$; $u(z,t)$ is the velocity of microbial mass $[LT^{-1}]$; $S_j(z,t)$ denotes the concentration of substrate $j, \mathbf{S} = (S_1, \ldots, S_m)$ $[ML^{-3}]$; $r_{M,i}(z,t,\mathbf{X},\mathbf{S})$ is the specific growth rate $[ML^{-3}T^{-1}]$; $L(t)$ denotes the biofilm thickness, free boundary $[L]$; $\sigma_a(t)$ is the attachment biomass flux from bulk liquid to biofilm $[LT^{-1}]$; $\sigma_d(t)$ is the detachment biomass flux from biofilm to bulk liquid $[LT^{-1}]$; D_j denotes the diffusivity coefficient of substrate j $[L^2T^{-1}]$; $r_{S,j}(z, t, \mathbf{X}, \mathbf{S})$ is the conversion rate of substrate j $[ML^{-3}T^{-1}]$.

Appropriate initial and boundary conditions are required to solve the previous system of nonlinear partial differential equations. In particular, at the substratum-biofilm interface $z = 0$ the condition of no concentration gradient is assumed for both the soluble and the particulate components. Equation 2.3 is derived from the mass balance of the *i*th microbial species set up for a control volume. Wanner and Gujer (1986) modeled the spreading of biomass as an advective mass flux of each *ith* species. In particular, the authors assumed that when in a control volume the net growth rate is positive and the biomass density remains constant, the biomass increases giving rise to a flux of biomass that crosses the control-volume's boundary. Equation 2.4 determines the velocity at which the microbial mass is displaced with respect to the film-support interface. The value of $u(z,t)$ is determined by the mean observed specific growth rate of the biomass and it is assumed identical for all species. Equation 2.5 defines the velocity at which the film-water interface moves; it depends on both the velocity at which the microbial mass is displaced, the velocity at which the biomass is exchanged between the biofilm and the bulk liquid and viceversa, here denoted as $\sigma_d(t)$ and $\sigma_a(t)$. In their work, Wanner and Gujer (1986) considered biomass loss only due to shear stress, modeled setting $\sigma_d(t) = \lambda L^2$ with λ constant, and sloughing by setting $\sigma_d(t)$ as a δ Dirac function. The model introduced by Wanner and Gujer (1986) can be classified as a free boundary value problem which is very complicated to discuss due to the concurrent presence of hyperbolic and parabolic partial differential equations. In addition, numerically speaking, it needs a special discretization scheme to consider the time-dependent change of the space domain (D'Acunto and Frunzo 2011, 2012; Szomolay 2008). The free boundary value problem contains two groups of nonlinear partial differential equations: the first system of n nonlinear hyperbolic partial differential equations describes the growth of microbial species in biofilms (2.3); the second group of m nonlinear parabolic partial differential equations governs the diffusion of substrates (2.6). The two systems are strictly connected like the biological processes they are aimed at modeling. Note that the time derivative in Eq. (2.6) is frequently neglected due to a standard time scale argument (Kissel et al. 1984), which leads to a hyerbolic-elliptic system of partial differential equations. The solution approach used

in (Wanner and Gujer 1986) is based on a coordinate transformation that eliminates the moving boundary by introducing the space coordinate $\zeta(t) = z/L$, which describes the distance from the substratum normalized by the biofilm thickness. This mathematical description of an idealized biofilm has been solved by a numerical solution technique based on the method of lines and has been addressed to some case studies. The existence of steady state solutions of this model applied to a single species biofilm was later proved by Pritchett and Dockery (2001).

Contemporary to Wanner and Gujer, Kissel et al. (1984) formulated a multispecies biofilm model able to describe the competition between microbial species for common substrates within a completely mixed continuous-flow reactor. This model is based on the same continuum approach used by Wanner and Gujer (1986) but does not include the loss of mass due to detachment. The two models differ in the way they describe the net growth of biomass at any position in the biofilm and, consequently, in the numerical treatment adopted for the moving boundary problem. When the biomass increases, the biomass density being kept constant, Kissel et al. (1984) hypothesized that the control volume increases in size (Fig. 2). Therefore, the numerical modeling of spatial variability in mass fractions and solute concentrations is accomplished by dividing the biofilm into a series of space elements with equal, but variable lengths. After each integration time step, the elements' lengths are recalculated, according to the volume expansion, or contraction obtained for the individual elements. All the equations have been solved numerically by using a fixed-step-size, fourth-order-accurate, Runge–Kutta technique. The model has been addressed only to dynamic state and no attempts have been made to solve the equations at steady-state conditions.

Rittmann and Manem (1992) combined the multispecies biofilm model developed in (Wanner and Gujer 1986) with the steady-state assumption. For a steady-state multispecies biofilm, it is assumed that the growth of all species, deriving from substrate utilization, is equal to all losses. The model is addressed to simulate the competition for space in a multispecies steady-state biofilm and to predict the steady-state substrate fluxes, the biofilm thickness and the species distributions deriving from specific bulk-liquid substrate concentrations. The model contains a set of ordinary differential equations, similar to the mass balance equations derived in Wanner and Gujer (1986). They are converted to be solved in a set of partial differential equations by introducing

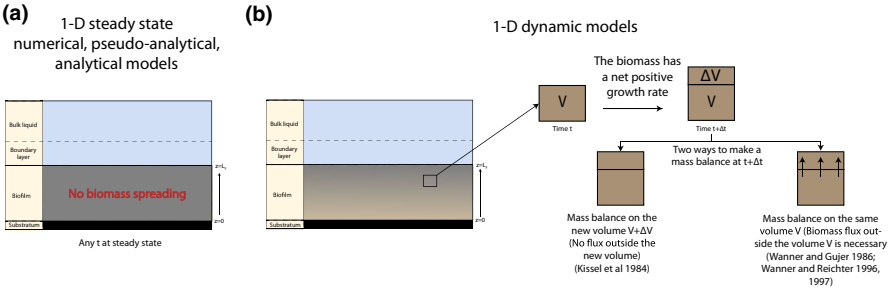

Fig. 2 1D continuum biofilm models: **a** steady-state models: the biomass distribution needs to be assumed a priori, **b** dynamic models: the spreading of biomass can be treated in different ways (figure adapted from Rittmann and Manem (1992))

the pseudo-time derivative, a means to perform the iterations required to achieve a correct steady-state solution.

The model introduced by Wanner and Gujer (1986) was later extended in (Reichert and Wanner 1997; Wanner and Reichert 1996), in order to simulate the effects of additional biofilm processes, as revealed by experimental observations. Therefore, the following processes which had been neglected in the previous model were taken into account: advective transport of dissolved components and diffusive movement of particulate components in the biofilm, the development of the biofilm liquid phase volume fraction, the transport of suspended solids within the pore volume of the biofilm and the exchange of cells and particles between the solid matrix and the pore volume, and the simultaneous detachment and attachment to the biofilm surface. In (Reichert and Wanner 1997), two new state variables are introduced: ϵ_l and θ. The first one represents the volume fraction of the liquid phase between the particulate components in the biofilm. The second one, also referred to as porosity, is introduced as the ratio of the volume between the biofilm solid matrix and the total biofilm volume. In (Wanner and Reichert 1996) the porosity and ϵ_l represented the same quantity since the transport of suspended solids in the pore volume is neglected. The two variables are related by the following equations (Reichert and Wanner 1997):

$$\epsilon_l + \sum_{i=1}^{nx} \epsilon_{P,Si} = \theta, \tag{2.7}$$

$$\theta + \sum_{i=1}^{nx} \epsilon_{M,Si} = 1, \tag{2.8}$$

where θ is the porosity; ϵ_l denotes the liquid phase volume fraction; $\epsilon_{P,Si}$ represents the volume fraction of the solids suspended in the biofilm pore volume; $\epsilon_{M,Si}$ is the volume fraction of the biofilm matrix components.

In both (Wanner and Reichert 1996; Reichert and Wanner 1997), particulate components are assumed to be transported not only by an advective flux, as stated in (Wanner and Gujer 1986), but an effective diffusive flux $J = -D_{M,i}\partial X_i/\partial z$ is introduced to describe the transport of cells and particles in the direction opposite to that of velocity $u(z,t)$. It is independent from microbial growth and accounts for the mixing of cells or particles in the biofilm solid matrix as a result of mechanical deformation of the matrix by hydraulic forces or bioturbation. The introduction of this diffusive flux modifies the nature of the Eqs. (2.3) which turned from hyperbolic to parabolic. ϵ_l is subject to an analogous advective flux $J = u\epsilon_l$ since it is assumed that the advective transport of particulate components does not change the ratio of liquid to solid phases in the biofilm. Moreover, to compensate the effective diffusive flux of particulate components, a flux of liquid phase in the opposite direction $J = -\left(\sum_{i=1}^{n} D_{M,i}/\rho_i \partial X_i/\partial z\right)$ is introduced and a production rate for the liquid phase volume fraction in the biofilm is formulated. Assuming the same notations of Eqs. (2.3)–(2.6) and considering $\rho_i \epsilon_{M,Si} = X_i$, $i = 1, \ldots, n$, the PDEs governing ϵ_l and X_i dynamics can be written as (Wanner and Reichert 1996):

$$\frac{\partial X_i}{\partial t} = -u\frac{\partial X_i}{\partial z} + \rho_i r_{M,i}\left(z,t,\mathbf{X},\mathbf{S}\right) - \frac{X_i}{1-\epsilon_l}\sum_{i=1}^{n} r_{M,i}\left(z,t,\mathbf{X},\mathbf{S}\right) + \frac{\partial}{\partial z}\left(D_{M,i}\frac{\partial X_i}{\partial z}\right) - r_{\epsilon_l}X_i,\ i=1,\dots n,\ 0<z<L\left(t\right),\ t>0, \tag{2.9}$$

$$\frac{\partial \epsilon_l}{\partial t} = -u\frac{\partial \epsilon_l}{\partial z} - \frac{\partial}{\partial z}\left(\sum_{i=1}^{n}\frac{D_{M,i}}{\rho_i}\frac{\partial X_i}{\partial z}\right) + (1-\epsilon_l)r_{\epsilon_l},\ 0<z<L\left(t\right),\ t>0, \tag{2.10}$$

where $D_{M,i}$ represents the effective diffusion coefficient of particulate components and r_{ϵ_l} denotes the production rate for the liquid phase volume fraction in the biofilm, which is modeled in order to simulate experimental data showing that in some cases porosity decreases from the biofilm surface to the substratum. The dissolved components are assumed to be transported in the liquid phase of the biofilm by a diffusive and advective flux, which is induced by a flux of water that derives from the transport of particulate components. The advective flux assumes a negligibly small value compared to the diffusive one. The PDEs for dissolved component concentrations write:

$$\frac{\partial S_j}{\partial t} = \frac{1-\epsilon_l}{\epsilon_l}u\frac{\partial S_j}{\partial z} + \frac{1}{\epsilon_l}\frac{\partial}{\partial z}\left(\epsilon_l D_j\frac{\partial S_j}{\partial z}\right) + \frac{1}{\epsilon_l}\sum_{i=1}^{n}\frac{r_{M,i}}{\rho_i}S_j + \frac{1}{\epsilon_l}\sum_{i=1}^{n}\frac{D_{M,i}}{\rho_i}\frac{\partial X_i}{\partial z}\frac{\partial S_j}{\partial z} + \frac{1}{\epsilon_l}r_{S,j}\left(z,t,\mathbf{X},\mathbf{S}\right),\quad j=1,\dots,m,\quad 0<z<L\left(t\right),\ t>0. \tag{2.11}$$

Moreover, the models (Reichert and Wanner 1997; Wanner and Reichert 1996) also take into account the simultaneous attachment and detachment of particulate components at biofilm surface, neglected in the original mixed-culture biofilm model where only the dominant process was explicitly modeled. More precisely, in (Wanner and Gujer 1986), the transport of cells and particles only occurs towards the biofilm surface and as a consequence, new attaching particulate material can only adsorb at the biofilm surface, but it cannot penetrate the biofilm. Modeling simultaneous attachment and detachment is possible only by taking into account the diffusive transport of particulate components which reproduces the mixing of cells and particles over the biofilm depth. The partial differential equations introduced in (Reichert and Wanner 1997) have been converted to a system of algebraic and ordinary differential equations and solved by the integration routines and numerical algorithms implemented in AQUASIM, a computer program designed for the identification and simulation of aquatic systems (Reichert 1994). A very detailed description of this simulation tool was provided in (Wanner and Morgenroth 2004).

Later, Rauch et al. (1999) introduced a comprehensive simplified model, whose approach consisted in decoupling the modeling of the diffusion process and spa-

tial distribution of bacteria species from the biokinetic reactions. This simplification derives from the need in faster, but rather accurate predictions, to avoid the computational efforts for solving the partial differential equations. The model is characterized by the following features. Diffusion is modeled as a steady-state phenomenon within each time step since substrate profiles are assumed to fast reach the equilibrium. The typical concentration boundary layer is neglected. The system is divided in two compartments: bulk liquid and biofilm. The biofilm is constituted by a liquid phase in which dissolved substances are transported by molecular diffusion and a solid matrix which is constituted by several bacterial species, particulate substrate and inert material. The concentration of particulates and density in biofilm are expressed by Eq. (2.8). Substrates are transported inside the biofilm by molecular diffusion; when they do not fully penetrate the solid matrix, the reaction is considered as diffusion limited and takes place only over a certain depth of the biofilm. According to Harremoes (1978), the volumetric reaction rate is assumed to be zero-order respect to the concentration of substrate S in the biofilm and the penetration depth is derived from an analytical solution to the diffusion equation. The model is solved by using a two-step procedure: (1) for each conversion process that is influenced by diffusion, the active fraction of the biomass within the biofilm is computed by means of the analytical solution to the diffusion equation; (2) all the conversions within the biofilm are then calculated assuming the biofilm as an ideally mixed reactor but only with the active fraction of the species contributing to the conversion process. The use of zero-order reaction rates is justified by the need of analytical solution for the substrate penetration depth that represents a basic concept for decoupling the diffusion and biokinetics reaction.

Despite their dimensionality, 1D biofilm models still represent an active topic in biofilm research area as proved by more recent models proposed in literature. In (Lee and Park 2007), the authors introduced a 1D mixed-culture biofilm model based on the hypothesis that each particulate component has different space occupancy within the biofilm according to its fundamental nature, such as size and density. In this work space occupancy is not defined as the reciprocal of component density, as stated in (Wanner and Reichert 1996), but this feature also takes into account the liquid volume that coexists within the biofilm solid matrix. Internal porosity is calculated by the composition of the particulate components, which changes during biofilm growth. The model is based on the same mass balance equations introduced in (Reichert and Wanner 1997; Wanner and Reichert 1996), but derived for the whole biofilm volume. The concept of effective diffusive transport is introduced and the model has been successfully applied to simulate the consolidation phenomenon. The partial differential equations have been solved by converting them into a system of ordinary differential equations in a dimensionless form, later solved by using the *ode15s* tool provided in MATLAB software.

Rittmann et al. (2002) reported a transient multispecies biofilm model (TMSBM) especially focusing on the kinetics of the growth related microbial products. This model represents a synthesis of the key modeling features used to describe multispecies biofilms and it is addressed to biofilms that experience time-varying conditions, particularly including periodic detachment by backwashing. The TMSBM contains non steady-state mass balances for each of the four types of biomass represented (2.3) and for the soluble species in a layer of biofilm (2.6). Similarly to (Wanner and Gujer

1986), it is assumed that the spreading of the biomass gives rise to a biomass-flux which involves a biomass velocity. This velocity represents the physical rate at which all types of biomass move into or out of a biofilm layer by crossing the layer's boundaries. The partial differential equations constituting the model are solved by using a separate-solution strategy. The calculations for the soluble species are separated from those for the biomass species in order to avoid the accumulation of rounding errors. Substrate and product calculations are performed for fixed biomass distributions, while substrate profiles are kept constant when biomass calculations are performed. This strategy allows us to reduce the computing load. To solve the biomass balance equations the TMSBM is based on a hybrid strategy between the approach used in (Wanner and Gujer 1986) and (Kissel et al. 1984). This new method allows biomass flux among equal size layers, which in turn can change whether the biofilm is growing, shrinking or attaining a steady-state. The sum of the growth and decay for all the biomass species in all the layers indicates the overall net growth. Based on this value and assuming a constant total biomass density, the size of each layer changes over time. Moreover, the model includes a new scheme for the net growth of each species in a single layer as related to the net growth of all species within the same layer and the adjacent layers.

Recently, D'Acunto and Frunzo (2011, 2012) have transformed the partial hyperbolic differential system (2.3) introduced in (Wanner and Gujer 1986) into an integral system by using a characteristic-like method, where the characteristics are the lines $z = s(z_0, t)$ defined by:

$$\frac{\partial s}{\partial t}(z_0, t) = u\left(s\left(z_0, t\right), t\right), \quad s\left(z_0, t\right) = z_0 \quad 0 \leq z_0 \leq L_0. \tag{2.12}$$

The same method was later applied for a qualitative analysis of the attached cell layer in multispecies biofilm formation (D'Acunto and Frunzo 2012). Compared to the free boundary problem introduced in (Wanner and Gujer 1986), this biological process is described by a free boundary problem for nonlinear hyperbolic equations where the initial biofilm thickness is set to zero. In this case, the free boundary is represented by a space-like line. An existence and uniqueness theorem of solution to the systems (2.3)–(2.6) was proved by the fixed point theorem (D'Acunto and Frunzo 2011, 2012; D'Acunto et al. 2016). The method of characteristics was also used for numerical purposes to simulate the dynamics of multispecies biofilms (D'Acunto et al. 2011; Mattei et al. 2015a, b). In the case of simpler single species systems, Abbas et al. (2012) formulated the free boundary value problem as an ordinary differential equation and investigated the longtime behavior of the Wanner–Gujer model assuming different detachment functions and incorporating the role of the hydrodynamic regime.

Klapper and Szomolay (2011) demonstrated by an exclusion principle that the Wanner–Gujer model (Wanner and Gujer 1986), evaluated under steady-state conditions, leads to restrictions on ecological structure since it neglects downward microbial motility. The introduction of a diffusion flux for motile species (Reichert and Wanner 1997; Wanner and Reichert 1996) may be able to negate the conditions leading to the exclusion principle. In a recent contribution, D'Acunto et al. (2015) have been able to

take into account the biological process of colonization of new species and transport from bulk liquid to biofilm (or viceversa) keeping the equations in hyperbolic form.

Parallel to the development of 1D dynamic models, more complex steady state models have been developed during the last decade. Pérez et al. (2005) developed a biofilm model based on the assumption of zero and first order kinetics in biofilms to compute substrate fluxes into the biofilm. More precisely, the approach which has been used is based on the weighted average of the analytical solutions for first and zero-order reaction kinetics. Compared to numerical models, the use of analytical solutions, apart from simplicity, allows the analysis of the effects that each term, or parameter can have on the overall flux. Beyenal and Lewandowski (2005) introduced a model able to reproduce biofilm heterogeneity by using a 1D continuum approach. The biofilm is modeled as composed by a finite number of layers characterized by different nutrient concentration, effective diffusivity and density. Each of these layers is modeled as a uniform biofilm and the effective diffusivity is recognized as the control parameter for space discretization. The model is aimed at quantifying mass transfer in layered biofilm as well as comparing the results with a homogeneous biofilm model. The effective diffusivity is expressed as a linear function of space coordinate z and biofilm density; its gradient is used to append the equation quantifying mass transfer in homogeneous biofilms by a factor representing biofilm heterogeneity. More recently, Gonzo et al. (2012, 2014) developed a new approach to model steady state activity of heterogeneous biofilm. The main difference with the work of Beyenal and Lewandowski (2005) lies on the fact that the new approach does not require numerical simulations.

2.3 Multidimensional continuum models

The development of multidimensional continuum models reflects the need of reproducing the complex morphology of biofilms, which arises from the interaction with the surrounding liquid and the dynamics of transport and consumption of substrates. These models are amenable to mathematical analysis and do not rely on *ad hoc* rules to simulate growth processes. For an easier orientation of the reader the multitude of multidimensional continuum models can be subdivided in two main categories based on their modeling goals: some of them are mainly concerned with biofilm growth of single-species or multispecies systems, while others have been mainly directed towards the implementation of biofilm deformation (Böl et al. 2009; Taherzadeh et al. 2012; Towler et al. 2007). The first category includes biofilm models mainly devoted to analyze the biological aspects of biofilm growth, without omitting the modeling of the hydrodynamics, which however has been based on simplifying assumptions concerning Navier–Stokes equations and that makes use of specific methods to solve the partial differential equations on irregular shape domains. Many of them are formulated as multifluid or one fluid multicomponent models. The second category incorporates fluid-structure interactions but neglects the biofilm growth aspect. This is justified by the natural difference in time scales between biofilm growth and deformation induced by the liquid flow. Most of these attempts at including mechanical aspects into biofilms seem to have been restricted to single-species biofilms and have been related to the

application of the immersed boundary method (Cogan 2007a; Vo et al. 2010; Dillon et al. 1996; Hammond et al. 2013, 2014; Klapper et al. 2002; Stotsky et al. 2015; Sudarsan et al. 2015). The description of such models is out of the scope of this work. Therefore, we just focus on the analysis of the multidimensional continuum models related to biofilm growth.

As stated in (Eberl and Demaret 2007), in the 1D case, dynamic biofilm models are mostly formulated as free boundary value problems and were based on the assumption that newly produced biomass is converted into new biofilm volume which moves according to a convective transport mechanism. The increasing biofilm thickness and the speed of propagation of the biofilm/liquid interface normal to the substratum can be calculated from the production terms by integration over the biofilm thickness. In the multidimensional case, this approach requires the introduction of an evolution equation for the convective biomass transport velocity. This equation can be derived by introducing the idea that biofilm growth generates a pressure field within the biofilm, which is responsible for the spreading velocity. Therefore, a further unknown variable (pressure) has to be modeled. To solve this issue different modeling approaches have been introduced: the description of the biofilm as a rigid/elastic/viscoelastic solid or highly viscous fluid has been carried out. Besides, material properties of the biomass have been explored by incorporating them in model equations to better understand biofilm structural stability.

The first attempt to model biofilm growth as a convective transport mechanism was introduced in (Wood and Whitaker 1998, 1999) where the authors developed a macroscopic description of microbial growth by using the sub-cell-scale information of mass transport and intracellular reactions. However, the mechanistic problem arising from the calculation of the convective field was solved only by introducing an *empiricism* which requires the experimental determination of a *growth coefficient*.

An alternative approach to the convective transport mechanism was introduced in (Eberl et al. 2001). The authors developed a spatio-temporal continuum model in which the biomass spreading is described by a nonlinear density-dependent diffusion mechanism (Fig. 3).

The model is aimed at describing hydrodynamics, transport and consumption of nutrients and biomass production for a single species biofilm. Biomass spreading

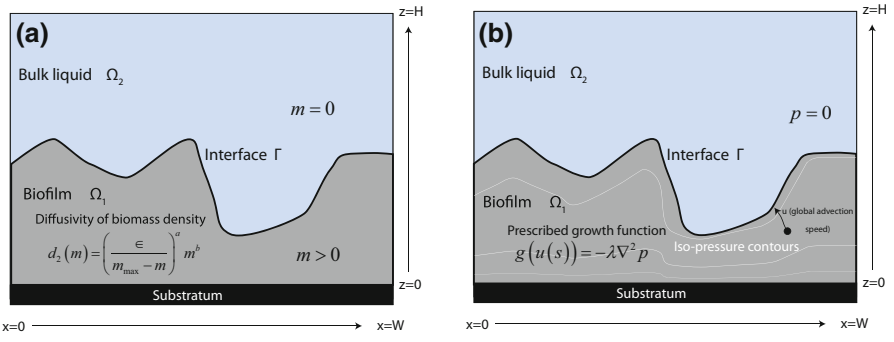

Fig. 3 2D continuum models: **a** spreading mechanism adopted by Eberl et al. (2001), **b** spreading mechanism adopted by Klapper and Dockery (2002)

occurs only when the biomass density $m(t, x)$ reaches a known a priori maximum value (waiting time behavior); as a consequence a density-dependent diffusion coefficient is introduced. The system is divided in two regions separated by an interface $\Gamma : \Omega_1$ that represents the liquid region and Ω_2, the solid biofilm region. The distinction between Ω_1 and Ω_2 is made by the biomass density $m(x, t) = 0$ or $m(x, t) > 0$, respectively. The model is governed by the following equations (Eberl et al. 2001):

$$\nabla \cdot \mathbf{u} = 0, \quad \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} = -\frac{1}{\rho} \nabla p + \nabla^2 \mathbf{u}, \quad \text{in} \quad \Omega_1 = \{x \in \Omega | m(t, x) = 0\}, \tag{2.13}$$

$$\mathbf{u} = \mathbf{0}, \quad \text{in} \quad \Omega_2 = \{x \in \Omega | m(t, x) > 0\}, \tag{2.14}$$

$$\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c = \nabla \cdot (d_1(m) \nabla c) - f(c, m) \quad \text{in} \quad \Omega_2 = \{x \in \Omega | m(t, x) > 0\}, \tag{2.15}$$

$$\frac{\partial m}{\partial t} = \nabla \cdot (d_2(m) \nabla m) + g(c, m) \quad \text{in} \quad \Omega_2 = \{x \in \Omega | m(t, x) > 0\}, \tag{2.16}$$

$$f(c, m) = \frac{k_1 c m}{k_2 + c}, \quad g(c, m) = k_3 (f(c, m) - k_4 m), \tag{2.17}$$

where $c(t, x)$ represents the nutrient concentration $[ML^{-3}]$; $m(t, x)$ is the biomass density $[ML^{-3}]$; $\mathbf{u}(t, x)$ is the flow velocity $[LT^{-1}]$; $p(t, x)$ is the pressure in the bulk region when the density and kinematic viscosity are kept constant $[ML^{-1}T^{-2}]$; ρ is the fluid density $[ML^{-3}]$; $f(c, m)$ is the Monod reaction term for nutrient consumption $[ML^{-3}T^{-1}]$; $g(c, m)$ is the biomass production and decay term $[ML^{-3}T^{-1}]$; $d_1(m)$ is the diffusion coefficient for nutrient transport $[L^2T^{-1}]$; $k_1, \ldots, k_4$ are parameters for biomass production and decay; $d_2(m)$ is the diffusivity of biomass density $[L^2T^{-1}]$ expressed by the following equation:

$$d_2(m) = \left(\frac{\epsilon}{m_{max} - m} \right)^a m^b. \tag{2.18}$$

The Eqs. (2.13)–(2.18) solve both the hydrodynamics and the biofilm evolution; they are strictly connected since the regions Ω_1 and Ω_2 both depend on $m(t, x)$. Biomass production is assumed to be established only by reaction kinetics and the biomass diffusivity is assumed to vanish as $m(t, x)$ gets small, but it increases as $m(t, x)$ grows thanks to biochemical reactions (2.15)–(2.18). The model (2.13)–(2.18) is mathematically complicated and difficult to be handled analytically. To solve it, the authors assumed hydrostatic state and introduced dimensionless dependent variables, since the major difficulties of the model derive from the Navier–Stokes equations (2.13). In this way the model reduces to a spatio-temporal predator prey model for biomass and nutrients. The model behavior has been validated only by numerical simulations carried out in 1D and 3D by using a finite difference scheme which is solved explicitly for the slower biomass spreading process and implicitly for the faster nutrient transport process. The numerical analysis is aimed at showing the sensitivity

of the biofilm behavior to crucial parameters and confirms that the model results are in good agreement with previous experimental and modeling experience.

Further analysis and application of the model introduced in (Eberl et al. 2001) were performed in (Duvnjak and Eberl 2006; Eberl and Demaret 2007; Eberl and Efendiev 2003). In these studies the authors focus on the evaluation of different numerical schemes able to handle the diffusion singularity effects arising from Eq. (2.18) or compute sharp travelling wave solutions with gradient blow up describing interface propagation phenomena (Jalbert and Eberl 2014). Analytical studies of the developed model were also worked out to demonstrate the existence and uniqueness of solutions (Efendiev et al. 2002, 2009). Eberl and Sudarsan (2008) later extended the degenerated diffusion-reaction model for biofilm growth and disinfection introduced in (Eberl and Efendiev 2003) to account for the convective transport of substrates in the bulk liquid (see Sect. 4.2). The model in (Eberl et al. 2001) was also extended to the case of multispecies systems (Eberl et al. 2010; Khassehkhan et al. 2009a; Muhammad and Eberl 2011) and has been applied recently to the case of quorum sensing induced dispersal in a 2D setting neglecting flow field calculations (Emerenini et al. 2015). For a general overview on existence and uniqueness of solutions of this type of models you can refer to (Sonner et al. 2015).

The concept of biofilm as a homogeneous, viscous, and incompressible fluid of constant density, satisfying Darcy's law was firstly introduced in (Klapper and Dockery 2002). In this pioneer work, the authors introduced an equation that regulates the state variable p (pressure) in the biofilm phase:

$$\lambda \nabla^2 p + g(u(S)) = 0, \tag{2.19}$$

where p is the osmotic pressure $[ML^{-1}T^{-2}]$; λ is the Darcy constant $[TL^3M]$; S is the concentration of the rate-limiting substrate $[ML^{-3}]$; g is a prescribed growth function $[T^{-1}]$; u is the substrate uptake rate $[ML^{-3}T^{-1}]$.

The pressure equation is solved in the biofilm region after setting specific boundary conditions: the aqueous region is supposed to be static near the biofilm surface and so a constant pressure is assumed for the bulk liquid (Fig. 3). Equation (2.19) is coupled with the solution of a nutrient diffusion-reaction mass balance, which provides the field of concentration S. The substrate is assumed to diffuse through the bulk region into the biofilm, where it also spreads and is consumed. The model does not take into account the internal chemical signaling for biofilm growth and behavior and the influence of fluid dynamics. The equations have been solved numerically on a uniform 2D rectangular grid. The biofilm-bulk liquid interface evolution is tracked by using the level set method.

The work of (Klapper 2004) represents the sequel of (Klapper and Dockery 2002) and examines the formation of biofilm fingers and mushrooms. According to Klapper et al. (2002), the hypothesis of biofilm as a viscoelastic fluid is adopted, but the analysis is restricted to the case of static or nearly static bulk fluid. Therefore the substrate is assumed only to diffuse from bulk liquid into the biofilm and the shear stress and the associated viscoelastic response is not considered. Under the hypothesis of incompressibility of the biofilm matrix and the assumption of Darcy law for the biofilm interface velocity, the continuity equation reduces to Eq. (2.19). According

to Klapper and Dockery (2002), the biofilm-liquid interface $z = h(x, y, t)$ evolution follows Eq. (2.20) (Klapper 2004):

$$\frac{dh}{dt} = -\left(\nabla p \cdot \mathbf{n}\right)\left(\hat{\mathbf{z}} \cdot \mathbf{n}\right). \tag{2.20}$$

The performed nonlinear analysis suggests that in the case of biofilms free of external mechanical stress, overall growth is inhibited by the presence of growing perturbations in the linear stage. A generalization of the previous 2D model (Klapper and Dockery 2002) and of the earlier 1D model (Wanner and Gujer 1986) has been proposed by Alpkvist and Klapper (2007) who developed a continuum model for the heterogeneous growth in biofilm systems with multiple species and multiple substrates. This model represents the early work of the rigorous mathematical treatment of continuum multidimensional multispecies biofilm models and it is based upon a combination of the approach introduced in (Wanner and Gujer 1986; Klapper and Dockery 2002). The domain is subdivided in two regions: the biomass region B_t and the liquid region Ω_{Bt} where Ω is defined as an open sunset of R^3. The domain has two moving boundaries: the biomass-liquid interface defined by the curve Γ_t and the bulk liquid interface at a fixed height Γ_{Hb} above B_t, defined by the curve Γ_{Hb}. In the region above the curve Γ_{Hb}, fluid mixing is able to replenish or remove diffusive components faster than they are used or produced. The model takes into account N_b different components or phases for the biomass region and N_c different substrates. The model consists of a series of partial differential equations derived on the basis of conservation laws and reaction kinetics. As in (Wanner and Gujer 1986) the transport of biomass is governed by an advective process characterized by a volumetric flow $\mathbf{u}(t, x)$ equal for all species. According to Klapper and Dockery (2002), the biofilm is modeled as a homogeneous, viscous, incompressible fluid with a velocity given by Darcy's law (Alpkvist and Klapper 2007):

$$\mathbf{u} = -\lambda \nabla p, \tag{2.21}$$

where $p = p(t, x)$ is the pressure $[ML^{-1}T^{-2}]$; λ is the Darcy constant $[TL^3M]$.

The model is based on semilinear Poisson equations for substrate concentrations (2.22), linear Poisson equation for pressure (2.23), and advective equations for the biomass volume fractions (2.24) (Alpkvist and Klapper 2007):

$$-D_j \nabla^2 C_j = r_j \quad j = 1, \ldots, N_c, \tag{2.22}$$

$$-\nabla^2 p = \sum_{i=1}^{N_b} \frac{g_i}{\rho_i^*} \quad i = 1, \ldots, N_b, \tag{2.23}$$

$$\frac{\partial \theta_i}{\partial t} - \nabla p \cdot \nabla \theta_i = \frac{g_i}{\rho_i^*} - \theta_i \sum_{i=1}^{N_b} \frac{g_i}{\rho_i^*} \quad i = 1, \ldots, N_b, \tag{2.24}$$

where C_j is the substrate concentration $[ML^{-3}]$; D_j is the assumed constant substrate diffusivity $[L^2T^{-1}]$; r_j is the substrate uptake rates $[ML^{-3}T^{-1}]$; g_i is the biomass

growth or loss rate $[ML^{-3}T^{-1}]$; ρ_i^* is the individual density for biomass components, assumed to be constant in time and space $[ML^{-3}]$; $\theta_i = \theta_i(t, x)$ denotes the volume fraction of the ith species.

Applied to a planar biofilm system the model reduces to a 1D model equivalent to the Wanner and Gujer system. Model simulations have been based upon accepted numerical methods with an existing error analysis. In particular, the time evolution of the biomass region is calculated by using a level set function as in (Klapper and Dockery 2002). The model has been used to simulate in 2D and 3D biofilm growth in growth-limited and transport-limited regimes. Basing on the 1D Wanner and Gujer modeling approach (Wanner and Gujer 1986) and its multidimensional extension (Alpkvist and Klapper 2007), Rahman et al. (2015) have recently developed a two-species cross-diffusion model, which reduces in the single species case to the density-dependent diffusion-reaction model introduced in (Eberl et al. 2001). The model is derived from mass and momentum conservation principles and introduces an alternative model closure which does not make use of the assumption of summing up to unity of the volume fractions adopted in (Alpkvist and Klapper 2007; Wanner and Gujer 1986), but it is essentially based on the introduction of an algebraic relationship which relates the pressure P driving bacterial movement to the biomass densities. In particular, for the dual-species system, the authors reduce the momentum balances to the Darcy like Eq. (2.25), by assuming the friction loss terms proportional to the biomass fractions of the interacting species X, Y and the velocity u and neglecting the inertial terms based on time-scale arguments as in (Alpkvist and Klapper 2007; Klapper and Dockery 2002; Merkey et al. 2009). Such equations are then solved for the moments uX and uY by assuming the pressures acting on each microbial species (P_1 and P_2) as distributed between both species proportionally, relatively to their current density (Eq. (2.26)). The terms uX and uY are then introduced into the mass balances for X and Y resulting in the cross-diffusion system (2.27):

$$\begin{cases} \nabla P_1 + f(X+Y)uX = 0, \\ \nabla P_2 + f(X+Y)uY = 0, \end{cases} \tag{2.25}$$

$$P_1(X,Y) = \frac{X}{X+Y}P(X+Y), \quad P_2(X,Y) = \frac{Y}{X+Y}P(X+Y), \tag{2.26}$$

$$\begin{cases} \frac{\partial X}{\partial t} = \nabla(D_{11}(X,Y)\nabla X + D_{12}(X,Y)\nabla Y) + G_1(X,Y)), \\ \frac{\partial Y}{\partial t} = \nabla(D_{21}(X,Y)\nabla X + D_{22}(X,Y)\nabla Y) + G_2(X,Y)), \end{cases} \tag{2.27}$$

where

$$\begin{cases} D_{11}(X,Y) = \frac{1}{f(X+Y)}\frac{\partial P_1}{\partial X}, D_{12}(X,Y) = \frac{1}{f(X+Y)}\frac{\partial P_1}{\partial Y}, \\ D_{21}(X,Y) = \frac{1}{f(X+Y)}\frac{\partial P_2}{\partial X}, D_{22}(X,Y) = \frac{1}{f(X+Y)}\frac{\partial P_1}{\partial Y}, \end{cases}$$

$G_{1,2}$ are the net biomass production rates.

Another deterministic approach to model biofilm growth was derived from material mechanics (Dupin et al. 2001). The biofilm is modeled as a continuous, uniform,

isotropic, and hyper-elastic material, whose expansion and deformation are governed by material stress–strain relations. The density is kept constant by deforming the biofilm matrix; this means that the pressure generated by cell division meets the resistance of the EPS matrix surrounding microbial cells.

A growing number of continuum multidimensional models based on the polymer solution theory and assuming the biofilm as an EPS-water polymer solution have been developed (Cogan and Keener 2004, 2005; Ehret and Böl 2013; Fowler et al. 2016a; Klapper and Dockery 2006; Tierra et al. 2015; Winstanley et al. 2010, 2015; Zhang 2012; Zhang et al. 2008a, b; Zhao et al. 2016b). In a pioneer work, Cogan and Keener (2004) introduced a mixture model based on the polymer-solvent two-phase theory, where the biofilm is treated as a hydrogel consisting of two immiscible materials, the networked produced polymers (EPS) and the fluid solvent (water). The gel undergoes swelling or contraction due to the absorption or discharge of solvent. The swelling is mainly affected by the chemical potential of the gel which is modeled as an osmotic or swelling pressure and depends on the structure of the polymers and the ionic environment. Negligible occupation volume for bacteria and substrates is assumed. The osmotic pressure is modeled by following the Flory–Huggins theory and physical forces due to the deformation of the matrix are separately taken into account. The network, which includes polymer, substrate and bacteria is modeled from a mechanical point of view as a constant density viscoelastic material while the solvent as a Newtonian fluid of much less viscosity. All the forces acting on the network and the solvent (surface forces, frictional drag, colligative force and hydrostatic pressure) are defined based on several constitutive relations and the momentum balance equations for both phases are derived. The mass conservation principle is applied to describe the network and solvent redistribution. An advection/diffusion/reaction equation is used to model the dissolved substrate dynamics. The equations modelling the growth of the bio-gel are expressed as follows:

$$\eta_n \nabla \cdot (\theta_n(\sigma_v + \sigma_e)) - h_f \theta_n \theta_s (\mathbf{U}_n - \mathbf{U}_s) - \nabla \Psi(\theta_n) - \theta_n \nabla P = 0, \tag{2.28}$$

$$\eta_s \nabla \cdot \left(\frac{\theta_s}{2} (\nabla \mathbf{U}_s + \nabla \mathbf{U}_s^T) \right) + h_f \theta_n \theta_s (\mathbf{U}_n - \mathbf{U}_s) - \theta_s \nabla P = 0, \tag{2.29}$$

$$\frac{\partial \theta_n}{\partial t} + \nabla \cdot (\theta_n \mathbf{U}_n) = g_n, \tag{2.30}$$

$$\frac{\partial B}{\partial t} + \nabla \cdot (B \mathbf{U}_n) = g_b, \tag{2.31}$$

$$\frac{\partial}{\partial t}(\theta_s c) + \nabla \cdot (c \theta_s \mathbf{U}_s - D \theta_s \nabla c) = -g_c, \tag{2.32}$$

where θ_n and θ_s denote the network and solvent volume fractions, which are assumed to sum to one; $\sigma_n = \sigma_v + \sigma_e$ is the network stress tensor, with σ_v and σ_e the viscous stress and elastic stress respectively; $\mathbf{U}_n$ and $\mathbf{U}_s$ are the network and solvent velocities; h_f is the constant friction coefficient; $\Psi(\theta_n)$ denotes the osmotic pressure which has been modeled through the Flory–Huggins theory and P defines the total hydrostatic pressure; g_n is the network production rate expressed as a function of bacterial concentration, substrate concentration and volume fraction of network; B

represents the bacterial concentration and g_b is the bacterial growth rate; c is the substrate concentration and g_c denotes the substrate utilization rate by bacteria. Note that from Eqs. (2.28)–(2.32) and considering $\theta_n + \theta_s = 1$, the volume averaged velocity expressed as $\mathbf{U} = \theta_n \mathbf{U}_n + \theta_s \mathbf{U}_s$ is not divergent free but is constrained to balance the network production.

A simplified momentum equation for the network is derived by assuming negligible frictional terms and neglecting the elastic stress in the evaluation of the surface forces. A consistency condition is derived for the interface between the biofilm and the solvent. An additional equation assumes non normal stress on the network at the interface. The linear stability of the simplified model was addressed and numerical simulations were used to explore the behavior of the model in the nonlinear regime. The results confirm the formation of mushrooming behavior under differential growth, in agreement with (Klapper and Dockery 2002).

The conceptual model of the biofilm as a biological gel consisting of EPS and water introduced by Cogan and Keener (2004) was later used by Winstanley et al. (2010), who adopted an explicit-non dimensionalization based on natural scales. Contrary to (Cogan and Keener 2004), they consider a momentum balance with negligible viscous stresses for the water on time scales larger than minutes. The model was studied in 1D and the existence of solutions was evaluated for travelling waves. In a recent work, the same authors have extended the model to investigate the biofilm ability of clogging a single pore space shutting off the fluid flow (Winstanley et al. 2015). The model accounts for a growing biofilm on the walls of a uniform long 2D channel and subject to erosion-like detachment. More recently, Fowler et al. (2016b), starting from the results of Winstanley et al. (2010), have analyzed the case of a biofilm growing in more than one lateral dimension. The model essentially consists in a free-boundary problem governed by non-standard type Stokes flow equations, which has been studied analytically and numerically. The results, both analytical and numerical, show the presence of cups on the interface in finite time. This suggests that the numerical method adopted by the authors and based on the hypothesis of a smooth interface would fail in the case of cups formation. As suggested by the authors, this issue could be solved by incorporating a surface tension as in (Cogan and Keener 2004).

Alternatively to the two fluid theory, a one-fluid two components formulation was introduced by Zhang et al. (2008a, b) in the phase-fields models, where biofilm is considered as an incompressible two-phase fluid with the two components expressed as volume fractions and playing the role of phase-field variables (Chen et al. 2015; Wang and Zhang 2012). As in (Cogan and Keener 2004), biofilm is assumed to be constituted by an *effective* polymer network including bacteria and EPS, and an *effective* solvent, which accounts for both pure solvent and nutrients. Contrary to (Cogan and Keener 2004), the average velocity is assumed to be divergent free and to govern the fluid motion. However, the polymer network velocity is assumed to differ from the average one by an excessive velocity which generates from the mixing of the two phases. The mixing flux is expressed as a function of the free energy variation and the Cahn–Hilliard equation (and its modification) has been adopted for the transport equation of the polymer network. A similar equation is adopted for the solvent fraction and an excessive solvent velocity is introduced. The model is completed with

the continuity equation for the average velocity, a momentum balance equation and the transport equation for the nutrient. In the momentum balance, the stress acting on the mixture has been derived by considering both the polymer and the solvent as viscous fluids, or by considering the dependence on the velocities of each component. More complex constitutive equations, namely the Rubber-elastic model and the Johnson–Segalman model, reproducing the elastic or viscoelastic behavior of the biofilm, could also be considered depending on the time scale of interest. The present treatment also provides a framework in which various constitutive relations for each constituent can be investigated in conjunction with the motion of the bulk fluid. The numerical scheme adopted to solve the model equations in 1D is a finite difference scheme.

Numerical simulations based on a similar finite difference scheme were performed in (Zhang et al. 2008b) to reproduce the growth, deformation and detachment of a biofilm colony in a 2D domain under different flow and shear conditions. In (Lindley et al. 2012), the model introduced by Zhang et al. (2008a, b), was applied to the case of a three component biofilm (the EPS network, bacteria and effective solvent) growing in a 2D domain and investigating the EPS-bacteria-flow interactions.

Duddu et al. (2008) proposed a continuum model to estimate substrate concentration, biomass advection velocity and biomass volume fraction and they later extended the model to fluid flow velocity field calculation (Duddu et al. 2009). The biofilm is characterized as a homogeneous isotropic elastic material constituted by two components, the active and the inactive biomass, while the fluid is assumed to behave as Newtonian with constant viscosity and in laminar flow. The fluid flow and the stress deformation problems are uncoupled under the hypothesis of small stress induced deformation (Picioreanu et al. 2001). The biofilm growth is supposed to be irrotational; therefore the velocity field is derived from a potential function. The system of partial differential equations governing the fluid hydrodynamics, substrate transport at steady-state, the mass balance for total biomass written in terms of the growth velocity potential and the mass balance equation for active biomass are solved by using the extended finite element method while the location of the biofilm/fluid interface is evaluated by the level set method.

In (Cumsille et al. 2014) a Hele-shaw type-like modeling was introduced: the modeled system is assumed to be composed by two fluids (biofilm and liquid) characterized by different viscosities and separated by a moving interface. The velocity field in the liquid compartment is assumed to be divergence-free due to incompressibility while the velocity field in the biofilm compartment is not divergence-free and Eq. (2.19) is assumed to hold. Compared to (Klapper and Dockery 2002), this work solves the pressure equation in the entire domain by imposing transmission conditions on the biofilm/bulk liquid interface, including the effects of the fluid motion induced by the evolution of the biofilm/liquid interface and accounts for advective substrate transport in and out of the biofilm. The mathematical problem has been solved by coupling the immersed interface method with the Level-Set method. Clarelli et al. (2013) have lately introduced a fluid dynamics model based on the *mixture theory* which considers the biofilm as a multiphase fluid. In contrast with most of the existing models, this work considers a finite speed of propagation for the hyperbolic equations. Existence

and uniqueness of global smooth solutions for the model applied to the 1D case have been proved recently (Bianchini and Natalini 2016).

3 Discrete models

Discrete models for biofilm research started to be developed in 1990*s*. Biofilms are assumed to be living systems inherently stochastic and researchers have been devising ways to express that stochasticity (Laspidou et al. 2010). They use approaches where the large-scale dynamics are emergent from the processes occurring at a small-scale and are generally defined bottom-up models since biofilm structure is not furnished as an input to the model but the complex morphology of biofilms emerges as a result of the actions and interactions of the biomass units with each other and the environment. The rules used to model interactions at a local level can be purely motivated through biological principles, rather than the analysis based on a mathematical and physical framework (Alpkvist et al. 2006). The basic idea consists in splitting the biomass accumulation and transport in two separate processes: the biomass growth kinetics are still governed by ordinary differential equations as for the continuum models, while the biomass transport mechanism is realized in a discrete way. Therefore, discrete models have been classified in three groups based on the biomass representation and the adopted spreading mechanism:

- *Cellular Automaton* (CA) models;
- Hybrid differential—discrete CA models, in which the mass transport is described by using differential equations while the biofilm structure development is treated by using a CA approach;
- *Individual-based Models* (IbMs).

In CA models, the biomass is represented in an array of small compartments (usually rectangular), as opposed to the agent-based representation of the IbMs that use particles located anywhere in space and characterized by essential state variables like cell mass and volume. CA models use volume averaging properties (density or concentration) as state variables for the biomass and they are so called biomass-based models. The three groups also differ on the biomass spreading rules used (see Sects. 3.1, 3.2, 3.3).

3.1 Cellular automaton models

Modeling biomass growth and spreading has been widely performed by using a CA approach (Barker and Grimson 1993; Chang et al. 2003; Colasanti 1992; Hermanowicz 1998, 1999, 2001; Picioreanu et al. 1998a, b; Pizarro et al. 2001, 2004; Tang and Valocchi 2013; Wimpenny and Colasanti 1997). CA models were originally developed for the Game of Life (conceived by the mathematician John Horton Conway in 1970) and were based on simple rules for building complex structures from simple and repetitive elements (Wimpenny and Colasanti 1997). In particular, the basic idea consists in miming the physical laws by a series of simple rules, easy to compute quickly and in parallel. More properly a CA model consists of a simulation which

is discrete in time, space and state (Ermentrout and Edelstein-Keshet 1993). Usually the model space is discretized in a grid of rectangular elements (often squares in 2D or cubes in 3D). Each grid element has four first-order neighbors and another four second-order neighbors in the 2D rectangular space discretization (Picioreanu et al. 2000a). The grid cell is allowed to fill up to a predetermined maximum and a simple rule-based system is employed to locate the extra biomass in a new compartment (Laspidou et al. 2010). Substrate diffusion is usually simulated by random walks of individual substrate particles; while biofilm growth is described as the multiplication of individual microbial cells when they consume substrate particles.

CA models can be divided in three classes: (1) deterministic or *Eulerian* automata; (2) *lattice gas* models; and (3) *solidification* models (Ermentrout and Edelstein-Keshet 1993). For the first model class, the spatial domain is divided into a fixed lattice and each lattice point has a state associated with it. The state at the next step is determined by earlier states of the cell and its neighbor. This type of CA model reproduces evolution equation with a partial differential equation or an integral equation. Lattice gas models are called particle systems and consist of a discrete spatial grid on which particles move and interact in some prescribed fashion. Solidification models resemble lattice gas models except for the concept of bound state.

The Diffusion-Limited Aggregation (DLA) models represent the first attempt to model the bacterial colony structures using a discrete approach (Fujikawa 1994; Fujikawa and Matsushita 1989; Matsushita and Fujikawa 1990; Tolman et al. 1989; Witten and Sander 1981). These models are based on the same grid system as standard CAs, but the array contains particles that can move among the squares in a prescribed pattern. They are based on an analogy between crystal growth and biofilm accumulation. In particular, DLA models assume that both crystallization and biofilm formation are driven by the mass transfer of some essential dissolved compounds from bulk liquid to a solid surface. These models are mostly focused on the important role played by the concentration gradients in the growth mechanism of bacterial colonies. The biofilm growth is assumed to be determined by the deposition of new layers of material on an existing surface. Dissolved matter diffuses through boundary layers; when it reaches a reactive surface, a surface reaction transforms it in solid phase. The basic idea of these models consists in choosing a seed particle as the origin of a square lattice on a plane. Biofilm growth occurs when another particle, released far from the origin and allowed to move randomly, reaches the nearest neighboring site to the origin and sticks to the site. Later these two particles are frozen in this position and another particle is released. Repeating this procedure, the cluster grows assuming in many cases an open and branched structure. DLA models are based on the simplifying assumption that nutrients diffuse only across a liquid boundary layer; actually nutrients also diffuse into the biofilm, leading to the appearance of a reaction zone in the bulk biofilm. This means that biofilm does not grow only at the surface but also in volume and the expansion of the solid–liquid biofilm interface is caused by the internal pressure generated by the growing biomass. DLA models have been applied to simulate the growth of bacterial colonies both at very low nutrient level on an agar plate and under higher nutrient concentrations. Although the shapes of DLA patterns may resemble those of certain bacterial colonies, the biological mechanism is clearly distinct since cells are added through division of nearby cells.

Later, Wimpenny and Colasanti (1997) developed a model that adds biological rules to DLA models. The stationary particle used in DLA models is replaced by a microbial cell. This microbial cell can occupy a single square and can produce copies of itself that will occupy neighboring squares. The cells consume resource units that can randomly diffuse over a predetermined range of neighboring compartments. In this model, growth occurs only if there is available free space in the neighborhood of the cell. This mechanism generates growth only in the outermost cell layer, just like in crystal formation and neglects any growth occurring inside the biofilm matrix. Moreover, the model does not take into account the conservation laws of the substrate amount converted into biomass. Despite these shortcomings, the model was able to demonstrate how changings in the concentration of a rate-limiting substrate can cause different morphology varying from dense structure to biofilms penetrated by water channels.

A quantitative CA model for homogeneous biofilms was introduced in (Pizarro et al. 2001). The main objective of this work was to link the stochastic CA parameters with the physical and kinetic parameters used in biofilm modeling in order to obtain quantitative predictions of macroscale activity. The CA model is presented as constituted by six different elements: lattice, cell, states, time, rules and neighborhood. The biofilm system is divided into two lattices: the first describing the spatial location of food particles, the second on the spatial location of the microbial particles that constitute the biofilm. In the substrate lattice, cells are subdivided into layers that represent the possible directions of displacement of substrate particles during diffusion. Each layer in the substrate lattice can have one of two states, describing absence (0) or presence (1) of food respectively. The number of food particles in a local neighborhood defines the concentration of substrate at that location. In the microbial lattice, the cells can assume three different states, absence (0), presence of one microbial particle (1), or presence of two microbial particles (2). The latter state describes the situation right after a reproduction event. The information on each lattice is updated at discrete time intervals. The dynamics of this update are governed by the CA rules, which represent the interaction of each cell and its neighborhood with the corresponding cells in the superimposed lattice. Each rule represents the application of the most important processes occurring in biofilm, namely diffusion, substrate utilization, bacterial growth, bacterial decay, and microbial distribution. The rules are applied to the two lattices in a sequential manner. Substrate diffusion is modeled by a random movement of food particles in the lattice. This movement is simulated in two steps, mixing and transport. Substrate utilization is modeled by introducing the probability that during a time interval, a microbial particle will consume a food particle. Microbial growth is modeled according to Wimpenny and Colasanti (1997). The probability of a microbial particle disappearing from the lattice at a given time step is evaluated by a first order coefficient which takes into account microbial decay and detachment. After the growth and decay steps, the microbial lattice is updated according to the biomass distribution rules: 1) conversion of a cell with two microbial particles into two cells with one microbial particle each; 2) elimination of the empty cells. The CA approach introduced by Pizarro et al. (2001) was later applied to incorporate the formation and decay of inert biomass and to include a self-organizing development of the biofilm structure (Pizarro et al. 2004).

3.2 Hybrid differential-discrete cellular automaton models

Hybrid differential-discrete CA are a class of models in which nutrient diffusion is modeled by using a differential equation usually assumed at a steady-state with respect to the bacterial growth, while the biomass spreading is treated by CA rules (Boraey et al. 2015; Hunt et al. 2003; Laspidou and Rittmann 2004b, a; Laspidou et al. 2005; Noguera et al. 1999b; Picioreanu et al. 1999, 1998a, b, 2000b, c, 2001). This type of model presents the same features of CA models, being thus characterized by the same drawbacks. However the use of finite difference methods for solving the nutrient field can lead to a faster and more realistic model solution (Picioreanu et al. 1998a).

A first attempt of combining continuous models with discrete ones to simulate complex biological structures, was introduced by Ben-Jacob et al. (1994), who developed a detailed model of bacterial colony growth using CA systems. The model includes the following generic features: diffusion of nutrients; movement of the bacteria; reproduction and sporulation; local communication. Nutrient diffusion has been modeled by solving a diffusion equation on a triangular lattice. Bacterial cells are divided in groups called "walkers" which can move on a triangular lattice within an envelope. Each walker is described by its location and an internal energy which affects its activity. The walker can loose or gain energy; when this energy drops to zero the walker becomes stationary while when this amount increases thanks to the consumption of nutrients and reaches a threshold, the walker duplicates. The model involves elements of cell to cell communication and chemotaxis and reflects some of the complexity of a microbial community.

Hermanowicz (1998, 1999, 2001) developed a 2D model in which biofilm is represented by a 2D array of "cells". Each model cell can be "occupied", i.e. occupied by the biomass, or "empty", i.e. filled with water; mathematically it is represented by a dynamic variable that changes according to prescribed rules. The work is aimed at demonstrating how the CA approach is able to model the formation of self-organized structures based on simple development rules on a small scale as well as it can evaluate the effect of the external environmental conditions. Model cells occupied by biomass will grow, divide or detach themselves according to a set of rules. Cell division depends on the probability of division, evaluated as a function of the environmental condition, such as nutrient concentration (Hermanowicz 2001):

$$P = \frac{c}{c + K} = \frac{(c/K)}{(c/K) + 1}, \tag{3.1}$$

where P is the probability of division; c is the local concentration of the limiting substrate $[ML^{-3}]$; K is the Monod half-saturation constant $[ML^{-3}]$.

A dividing grid cell spawns a daughter cell which will occupy one of the eight neighboring grid units with the following rules: if the grid cell is empty, the daughter grid cell will occupy it; if there is more than one empty cells, the choice is random; if all the neighboring cells are occupied the shifting occurs in the direction of least resistance. This direction is evaluated calculating the shortest distance from each occupied grid cell to the biomass interface. In this case the daughter cell will push a whole line of cells in the direction of the nearest biofilm surface, to find some room. This mechanism of

displacement resembles the concept of biomass advective flux formulated by Wanner and Gujer (1986), but in this case the biomass does not move with a uniform velocity, but it jumps in a stochastic manner. Grid cells consume substrates that diffuse inside the boundary layer, whose thickness is a model input, and the biomass, while substrate concentrations remain constant outside the boundary layer. A matrix representing nutrient concentrations is superimposed on the working space containing water and biomass. The concentration field can be described by a Poisson equation. This equation is not solved numerically, but an analytical solution is introduced by modifying the one obtained for 1D biofilm and zero-order nutrient uptake kinetics (Hermanowicz 2001):

$$c = \left({c_S}^{1/2} - \left(\frac{k}{2D} \left(\frac{1}{8} \sum_{i=1}^{8} \frac{1}{d_i^2} \right)^{-1} \right)^{1/2} \right)^2, \tag{3.2}$$

where C_S is nutrient concentration maintained constant outside the boundary layer $[ML^{-3}]$; k is the uptake rate for zero-order kinetics $[ML^{-3}T^{-1}]$; d_i is a penetration distances $[L]$.

Based on the idea that the resistance to mass transport is a function of the penetration distance, Eq. (3.2) is derived by modeling the overall resistance as a harmonic means of the resistances evaluated in the eight directions of the nutrient supply considering each point inside the biofilm. Detachment occurs randomly at a fluid/biomass interface with a probability increasing proportionally with the biofilm thickness. More precisely, the probability of cell erosion is evaluated as a function of the hydrodynamic shear stress and biofilm cohesion. That approach allows the researchers to consider the detachment of larger clusters.

Picioreanu et al. (1998a) introduced the so defined first hybrid-differential approach suitable for modeling sessile cells, growing in a gel matrix. The model is still based on a CA approach for biomass spreading, but it evaluates the substrate field by solving a common reaction-diffusion equation. This model is meant to overcome a recurrent drawback of CA models deriving from the use of abstract parameters such as units of resource or random-walk distance. It relies on physical/chemical/biological parameters commonly used to describe biofilm systems (yields, concentrations, rates, fluxes of nutrients). Moreover, the combination of differential with discrete models allows the authors to predict the correct time evolution of biofilm growth, concentrations, fluxes and conversion rates, despite the typical CA algorithms which work in a completely abstract time and space. In this pioneer work, the state of the system is represented by using two variables: the soluble limiting substrate concentration and the biomass density, coupled to a matrix which stores information about the grid occupation. The model takes into account the three main processes characterizing biofilm development in hydrostatic conditions (i.e. diffusion-reaction-growth) and it is aimed at demonstrating the validity of the new combined differential-discrete approach in studying biofilm development. Substrate transport occurs only by diffusion through a concentration boundary layer and further on into the biofilm matrix. It is expressed in dimensionless form by the following equation (Picioreanu et al. 1998a):

$$\frac{\partial S}{\partial t} = \frac{D}{d^2}\left(\frac{\partial^2 S}{\partial X^2} + \frac{\partial^2 S}{\partial Y^2} + \frac{\partial^2 S}{\partial Z^2}\right) - \rho_S\,(C, S)\,, \tag{3.3}$$

where $S = c_S/c_{S0}$ is the dimensionless substrate concentration and c_{S0} is the substrate concentration in the bulk liquid; $C = c_X/c_{Xm}$ is the dimensionless biomass concentration and c_{Xm} is the maximum biomass density in a colony; D is the diffusion coefficient $[L^2T^{-1}]$; d is the characteristic length (in this case the bead diameter) $[L]$; $X = x/d, = y/d, Z = z/d$ are the space coordinates in dimensionless form; $\rho_S(C, S)$ is the normalized rate of substrate consumption $[T^{-1}]$.

The solution of the diffusion-reaction eqnarray is uncoupled from the calculations regarding the slower process of biomass spreading. In particular, Eq. 3.3 is solved by using relaxation algorithms and maintaining the matrices of biomass density and occupation state at a frozen level.

The biomass density is evaluated by solving the following equation (Picioreanu et al. 1998a):

$$\frac{\partial C}{\partial t} = \rho_X\,(C, S)\,, \tag{3.4}$$

where $\rho_X(C, S)$ is the normalized rate of biomass accumulation $[T^{-1}]$.

The occupation matrix is updated after solving the biomass balance. In particular, the biomass is redistributed when the maximum density is achieved in an elemental volume (x, y, z). The biomass is divided in two equal parts, redistributed in the neighboring space with no preferential direction according to simple CA rules (Fig. 4). The pressure exerted by the biomass growing in the biofilm depth generates displacement of cells towards the biofilm-liquid interface. A single-cell release mechanism for detachment is just to be considered for the biomass located outside the carrier sphere.

The model introduced in (Picioreanu et al. 1998a) was later applied to simulate the biofilm growth on solid flat surfaces (Picioreanu et al. 1998b). Despite the continuum models, biofilm structure properties such as shape, porosity and density must not be provided as input data, being generated by the model itself. Simulations at different substrate conversion/ transport rate ratios were performed to evaluate their effect on biofilm structure. The biofilm surface shape was characterized by using statistical quantities, such as biofilm surface enlargement, roughness, fractal dimension of biofilm surface. Biofilm structure complexity instead was evaluated like solids hold-up and biofilm compactness.

An extension of the previous model was presented in (Picioreanu et al. 1999, 2000b, c), taking into account the biomass growth and spreading, the diffusive and convective transport and transformation of substrates as well as the flow around the biofilm structure. The 2D model is fully quantitative, being based on first principles as Navier–Stokes equations, substrates mass balances and kinetic laws for biomass growth. The biomass growth and spreading is modeled following the approach introduced in (Picioreanu et al. 1998a). The mass balance of the substrate is modeled by a convection-diffusion-equation and the flow field is governed by the incompressible Navier–Stokes equations in laminar regimes. The flow field and biofilm shape are interdependent since flow field shears the biofilm surface, it erodes the

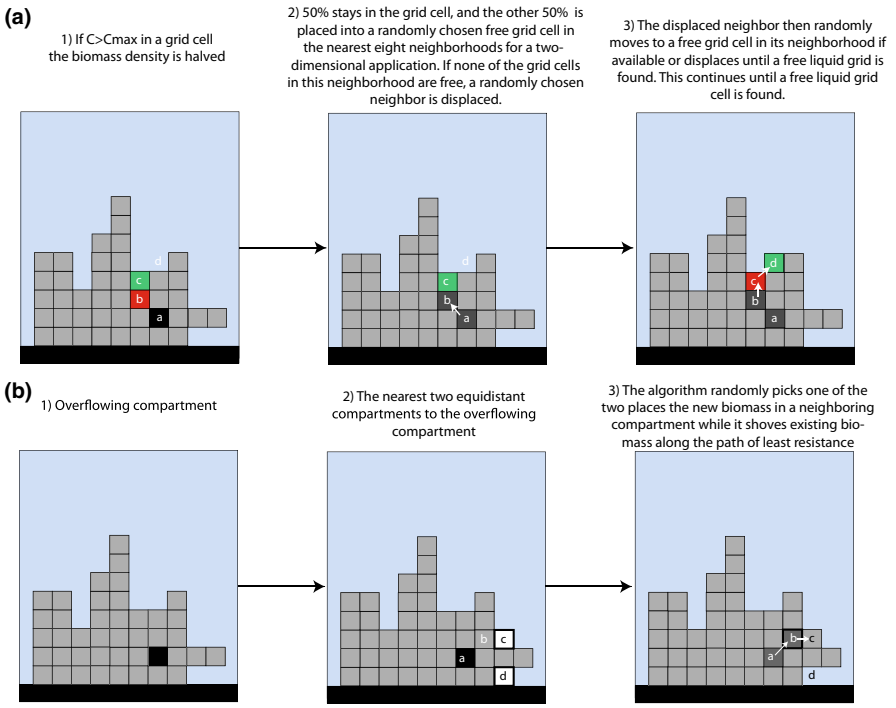

Fig. 4 Schematic representation of the spreading rules adopted in **a** Picioreanu et al. (1998a, b), **b** Laspidou and Rittmann (2004a, b). Figure adapted from Tang and Valocchi (2013) and Laspidou et al. (2010)

protuberances and it regulates the substrate concentrations at the biofilm-liquid interface. Simultaneously, changes in the biofilm shape determine a new boundary condition for the flow field and consequently, a different flow and substrate concentration. In this work, a new strategy to model biofilm development in time is presented. This technique is based on the idea that there is a clear separation of time scales in the biofilm growth. Therefore, each process is solved assuming all the other processes occurring at different time scales at steady state.

Detachment was incorporated later on into the hybrid discrete-differential approach previously described (Picioreanu et al. 2001). In this work, two known biofilm detachment mechanisms, i.e. erosion (loss of small biofilm parts—eventually only cells—mainly from the biofilm surface) and sloughing (loss of massive biofilm chunks, often broken from the substratum surface), are modeled in a unitary way assuming that detachment is caused by the stress developed on the biofilm structure (Van Loosdrecht et al. 2002). The authors assumed that the biofilm detachment results from the combined effect of liquid shear and biofilm strength. The liquid flow above the biofilm exerts forces on the biofilm structure, both in normal and tangential to its surface, so the biofilm structure is subjected to a stress state. The biofilm is assumed to be a homogeneous, isotropic, elastic material in the state of plane strain and the criterion of maximum distortion energy will be applied to evaluate where the biofilm breaks. In particular biofilm breakage is supposed to occur when the equivalent stress, expressed as a function of the normal and shear stresses, exceeds the cohesion strength.

Similarly to (Picioreanu et al. 1998a), Laspidou and Rittmann (2004a, b) developed a multi-com ponent cellular automaton model which combines the discrete representation of the solid phase by CA with classical continuous methods for soluble components. The model is based on the theory developed and quantified in Laspidou and Rittmann (2002a, b) and considers three solid species including bacteria, EPS and inert residual biomass, two soluble microbial products, one limiting-growth substrate and an electron acceptor. They are all quantified in dimensionless form according to Picioreanu et al. (1998a). The model reproduces in a 2D domain the growth of active biomass, the EPS and utilization-associated product formation, the EPS hydrolysis to biomass-associated products and their utilization as electron donors as well as the endogenous decay of active biomass to residual dead cells. The same solution strategy of Picioreanu et al. (1998a) is used to solve the substrate field, but two new concepts are introduced for the cellular automaton algorithm: the composite density $CompDen^{i,j}$ and the biofilm consolidation which describe the increase in biofilm density occurring over time, deeper in the biofilm. The composite density varies with time and space and it is calculated for each CA cell according to Eq. (3.5):

$$CompDen^{i,j} = X_a^{i,j}\chi_{a,max} + EPS^{i,j}eps_{max} + X_{res}^{i,j}\chi_{res,max} \tag{3.5}$$

where $X_a^{i,j}$ is the dimensionless density of the active biomass in *i,j*th cell; $X_{res}^{i,j}$ is the dimensionless density of the true residual inert biomass in *i,j*th cell; $EPS^{i,j}$ is the dimensionless concentration of EPS *i,j*th cell; $\chi_{a,max}$ is the maximum active biomass packing density [ML-3]; $\chi_{res,max}$ is the maximum of the true residual inert biomass packing density [ML-3]; eps_{max} is the maximum EPS packing density $[ML^{-3}]$.

Each of the solid-phase components is computed from mass-balance equations and redistributed according to the CA algorithm except for the residual inert biomass, which is only expected to accumulate at the bottom of the biofilm. The spreading of active biomass and EPS is simulated as the division of mother cells in daughter cells. In particular, the excess biomass is redistributed from one cell or compartment when the composite density exceeds a maximum value. The maximum composite density is specific for each compartment and increases over time with bioage (age of each biofilm department) in order to simulate the consolidation phenomenon. A consolidation ratio is calculated for each compartment as an exponential function of biofilm age. It represents the degree of maximum packing density. The excess biomass is redistributed when the sum of the dimensionless density of the solid-phase components exceeds the consolidation ratio. The model distributes the excess biomass by identifying the shortest or least resistance path (Fig. 4). Moreover, a first-order detachment law is included for the outmost layer of the biofilm. The outputs of the UMCCA model were later used to perform the biofilm stress analysis aimed at evaluating the biofilm's strength and resistance to detachment (Laspidou et al. 2005). Recent contributions related to the application of the UMCCA model deal with the calculation of the biofilm mechanical properties evolving with deformation (Laspidou et al. 2012, 2014).

3.3 Individual based models

The term IbMs is addressed to a class of multidimensional models whose objective is to describe the actions and properties of the individuals constituting the bacterial population or community (Ferrer et al. 2008; Kreft et al. 2001; Picioreanu et al. 2004a; Xavier et al. 2005b). IbMs use a bottom-up approach; they can also be classified as spatially structured population methods. This type of models was developed with the aim of overcoming all the drawbacks deriving from the application of discrete rules to biofilm spreading, which are typical of the CA approach (Eberl et al. 2001). IbMs allow cells movement only on a continuous set of directions and distances while CA models typically require that biomass moves only in the finite number of lattice directions. Transport and reaction of a solute species, local microbial growth rates are usually modeled using differential approaches (Xavier et al. 2004b). In all IbMs, bacterial cells represent the fundamental entities and they are modeled as hard spheres in continuous 3D space, each of them having a variable volume, mass and a set of mutable growth parameters. These spherical agents act independently, analogously to how individual bacterial cells behave within biofilms. IbM models do not specify any global (population level) laws such as exponential population growth. The behavior of the agents is defined explicitly with a set of rules that mimic the behavior of individual bacterial cells, i.e. growth through the consumption of substrates, reproduction through cell division, production of metabolites etc.

The first attempt to model bacterial colony growth by using this approach was proposed in (Kreft et al. 1998, 1999) and it was later used to simulate a multispecies biofilm (Kreft et al. 2001). The use of IbM for biofilms can be classified as a more realistic approach that quantitatively incorporates the physiology of individual cells (Hellweger and Bucci 2009). In (Kreft et al. 2001) the authors introduced a fully quantitative IbM based on BacSim, which consists of two main parts: one deals with the simulation of the growth and behavior of individual bacteria as autonomous agents; the other one deals with the simulation of substrate and product reaction and diffusion. According to Picioreanu et al. (1998a), the bacterial growth has been simulated assuming the diffusion process at a pseudo-steady state (between each biomass spreading iteration) since biofilm growth is usually a much slower process than diffusion of substrates into the biofilm. Each cell performs "actions" as a result of the environmental conditions and its internal state. It grows by consuming the substrates and divides when a certain volume is reached; it moves as a consequence of being pushed by its neighbors (Xavier et al. 2004a). The model considers the random variation of cell parameters, the maximal uptake rate and the volume-at-division, using a Gaussian distribution with a coefficient variation (CV) of 10%. The pressure buildup due to the growth of biomass is released by maintenance of a minimum distance among the neighboring cells. For each cell, the vector sum of all positive overlap radii with the neighboring cells is calculated and then the position of the cell is shifted in the direction opposite to this vector. Therefore, the biomass packing in the biofilm is defined by the shoving parameter K_{shov} which represents the spacing among cells. When a cell reaches a critical volume, it splits resulting in the creation of another cell, the "daughter", and the mass of the original cell is slightly unevenly distributed between these two spheres. The random choice of the direction for the placement of daughter cells and

the uneven division of mass between cells makes the model stochastic. The substrate concentration is governed by a reaction-diffusion equation which is solved by using a relaxation method. The uptake rates calculated for each bacterium occupying a certain grid element of the substrate field are averaged on an area percentage basis. Despite the unilateral shoving mechanism adopted in (Kreft et al. 1998), Kreft et al. (2001) introduced a mutual mechanism which minimizes the effect of a bias by making the shoving independent of the bacteria access sequence. This result is achieved by inverting the shoving sequence every 10 steps. The performed simulations revealed that the mutual scheme is better when avoiding overlaps and relocating cells and as well as it reduces sequential execution biases. The BacSim framework was compared with the population-level model introduced by Picioreanu et al. (1998a, b). The simulations show similar results in principle, as they model the same physical processes, but they differ in details of biofilm shape and growth of minority species. In particular, the IbM method better fits to the description of multispecies systems since CA models determine the production of excessive internal species mixing within a colony or the generation of anisotropic colonies.

The IbM approach introduced by Kreft et al. (2001) would be used later on to model the mechanism of production and spreading of EPS (Kreft and Wimpenny 2001a, b) and to test several evolutionary and ecological hypotheses (Kreft 2004). In (Kreft and Wimpenny 2001a), the EPS formation is stoichiometrically coupled to growth; the EPS produced is first bound to the bacterial agent forming a protective layer and then excreted as a separate agent that will participate in the shoving mechanism along with the bacterial agents. In (Kreft 2004) the IbM approach was applied to study the development of altruistic behavior by bacteria in biofilms. The extremely detailed level of biofilm description characterizing IbMs can represent a disadvantage in modeling systems with large-scale heterogeneity. In order to extend the spatial scale of the previous IbMs, Picioreanu et al. (2004a) introduced a multidimensional particle-based modeling approach which considers the presence of larger biomass particles, but keeps the rules for biomass redistribution and shoving introduced in (Kreft et al. 2001). In this model the biofilm biomass is divided in spherical particles containing only one type of active biomass and a fraction of inert biomass resulting from the decay of all active biomass types. The size of these spherical agents is chosen to represent a cell cluster of similar cells and no variability in metabolic parameters is included for all biomass particles of the same type. Biomass division and spreading is based on the same mechanism introduced in (Kreft et al. 2001). A simplified biomass detachment model is introduced: it consists of removing every particle, which is shifted above an imposed biofilm thickness limit, due to a shoving step. The substrate field is governed by a dynamic-state diffusion reaction equation, which is uncoupled from the solution of biomass evolutions, as stated in (Picioreanu et al. 1998b). A steady-state solution of the partial differential equation for mass balances of soluble substrates in the biofilm is found by a nonlinear multigrid algorithm. The numerical simulations reveal that the IbM framework for cell transport describes its continuum counterpart at least in a 1D case.

The modeling approaches introduced in (Kreft et al. 2001; Picioreanu et al. 1998a, b, 2004a), were integrated to provide a framework that defines the structure for the multi-dimensional, multispecies dynamic modeling of biofilm systems (Xavier et al. 2005b).

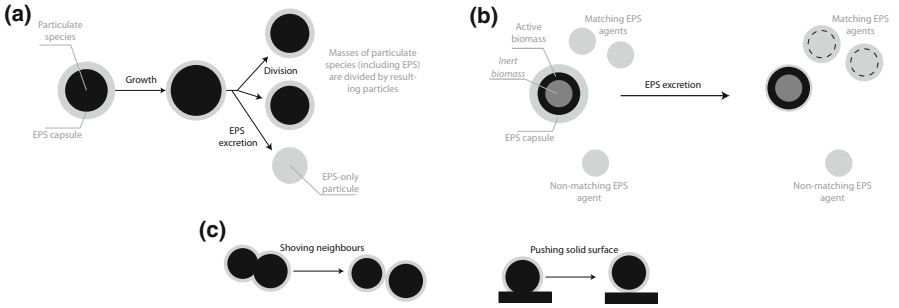

Fig. 5 Spreading mechanisms adopted by: **a**, **c** Xavier et al. (2005b); **b** Lardon et al. (2011). Figure adapted from these two papers

This IbM takes into account the concept of structured biomass which is constituted by multiple bacterial species, inert biomass and EPS. Three spatial scales are considered: a) individual scale, which deals with the behavior of biomass agents; b) biofilm scale, which works on a community level; c) system scale, which takes into account the interactions between the bulk liquid compartment and the biofilm. According to Picioreanu et al. (2004a), biomass particles can represent either a single cell or a cluster of cells of the same species. Each biomass particle is correlated to a pDocument which defines the number and type of particulate species constituting the agent. The mass of each particulate species varies according to a bioconversion equation and determines changes in the agent volume. The agent duplicates when the maximum particle radius is reached. The masses of all particulate species contained in the dividing agent are then redistributed between the two resulting agents. The EPS production and excretion are modeled according to Kreft and Wimpenny (2001a) (Fig. 5). In the case of EPS decay or inactivation of the bacterial biomass, the framework includes net reduction of the biofilm volume. A multidimensional extension of the method used in (Wanner and Gujer 1986) is introduced to study detachment and other biomass losses. In particular, a continuous detachment speed function is used to model both erosion and sloughing, as extensively described in (Xavier et al. 2005a) The computation of solute concentration fields is decoupled from biomass dynamics, as adopted in (Kreft et al. 2001; Picioreanu et al. 1998a, b, 2004a). The solute concentration field is computed by a multigrid solver, as previously applied in (Picioreanu et al. 2004a). Bulk concentration of solute species can be: constant, in which case the bulk liquid is assumed to be an infinite and constant solute supply; intermittent, by alternating feast and famine cycles, or can be computed from a mass balance equation applied to the whole system.

An alternative method to treat EPS was introduced in (Alpkvist et al. 2006). In particular, the authors used a continuous representation of EPS combined with an IbM of individual bacteria. According to Alpkvist et al. (2006) and Klapper and Dockery (2002), EPS is modeled as a viscous fluid, which is well justified by both experimental facts and physical grounds. On the other hand, the IbM approach is used to model the behavior of each bacterial cell, the local interactions between different microbial species and individual variation of microbial cells. The movement of EPS and cells on a global level in the biofilm is governed by an advection speed which is assumed

to follow the Darcy's law. At the same time, the individual cells (biomass spheres) undergo a shoving mechanism when they get too close to each other; the cell shoving introduces small local deviations in the flow field. The model has been applied to study the consolidation process in mature biofilms. This process seems to derive from the presence of a negative pressure in the lower region of the biofilm which is generated by EPS and cell degradation processes and results in cell transport towards the substratum.

A new modeling platform dedicated to IbM of microbial communities has been introduced recently by Lardon et al. (2011). In this work, the authors tried to combine most features of the previous models incorporating various improvements in order to provide a common basis for further developments. To address this aim, an open-source software called individual-based Dynamics of Microbial Communities Simulator (iDynoMiCS) was developed. The iDynoMiCS structure emerges from the combination of the previous modeling approaches but presents inherent differences. Primarily iDynoMiCS allows for the introduction of non-bacterial agents (archea, protozoa, algae or fungi). Microbial agents are structured in compartments including all intracellular components (active or inactive biomass, storage compounds, etc.), bounded by an outer layer of capsular EPS. All the agents are updated in a random order, which is changed for each time step in order to remove any bias. An individual agent can carry out a different suite of reactions compared to other individuals of the same species. The EPS excretion is represented by a particulate method: the EPS produced is continuously released into the environment and distributed to the same EPS particles, that are present in the neighborhood of the EPS-producing agent. Should those agents not be found, a new EPS particle must be created. To recreate a continuum representation of EPS, smaller radii of EPS particles are adopted (Fig. 5). According to Alpkvist et al. (2006) and Klapper and Dockery (2002), a pressure field is introduced to model biomass spreading or consolidation. As all IbMs, iDynoMiCS is affected by stochasticity in the choice of the initial agent locations and masses, the cell division threshold volume, the cell death threshold volume, the daughter cell's orientation and size, the excretion direction of new EPS particles and the updating order of the agents. During the last decade, the IbM approach has been widely used to predict several structural features of microbial biofilms and the results matched the experimental observations. The IbM approach was used to evaluate the biomass production/consumption and transport of biofilm for microbial fuel cells (Picioreanu et al. 2007a). The effect of microbial motility on biofilm morphology was analyzed in (Picioreanu et al. 2007c) and the concepts of IbM were applied to describe and optimize a biofilm and granular reactor (Xavier et al. 2007). The iDynoMiCS package has been integrated recently with three detachment mechanisms reproducing the effect of shear detachment on smoothening biofilms, nutrient-limited detachment on biofilm hollowing and erosion detachment on isolating bacterial clusters (Li et al. 2015).

Fozard et al. (2012) proposed a generic IbM to evaluate the effect of QS inhibitors on a developing biofilm. The model is based on a simplified technique for biomass spreading which makes use of voxels, cubic sub-compartments containing particles, substrate and signaling molecules. The voxels are also used to discretize the concentrations of dissolved compounds, which are to be assumed uniform within each voxel. The particles are modeled according to Picioreanu et al. (2004a) for what concerns growth and division and are located randomly within the voxels, only for visualization pur-

poses. Additional stochastic rules are added to model the switch from down-regulated to up-regulated state. Spreading within a single voxel is neglected, while particles can be exchanged based on the difference in pressure p_e among the neighboring voxels. The pressure p_e and the number of cells exchanged among the voxels are evaluated by using newly introduced equations which depend on the maximum number of particles in a voxel and a transfer coefficient. The direction in which each particle is displaced is chosen randomly.

Recently, a particle-spring approach has been used to take into account the variable cell shape and the aggregate morphology for simple single and mixed-population systems (Storck et al. 2014).

For the similarities with IbMs in considering cells as individual agents, it is worth citing the work of Tatek and Slater (2006), who introduced a 2D simulation model for biofilm formation and development on a flat surface. The model is based on the bond-fluctuation algorithm which allows the researchers to take some mechanical aspects of the cell membrane directly into account and it makes use of a square lattice where the single monomers, constituting the cell membrane, are able to move according to prescribed local rules (i.e. volume exclusion and Metropolis energy tests). Conversely to the differential approaches conventionally adopted by IbMs for transport and reaction of solute compounds, Tatek and Slater considered substrate particles as single monomers juming on the square lattice following specific rules and crossing cell membrane with a certain probability to mime the cell uptake. The state of each cell is individuated through the current age, the number of monomers constituting the cell membrane and the internal content of nutrient particles which represents the cell level of nutrition. These parameters play a crucial role in the simulation of the cell growth, division and death. Cell-wall and cell-cell interactions have been taken into account as well. A new version of the model which explicitly takes into account the synthesis and physical properties of the EPS was proposed in (Tao and Slater 2011), where EPS polymers were modeled as linear chains of monomers attached randomly on cell membranes. Recently the dissipative particle dynamics (DPD) approach has been adopted to simulate the growth and deformation of a biofilm in a flowing fluid (Xu et al. 2011). The flow field is governed by the incompressible Navier–Stokes equations whose solution is approximated by the low Mach number flow of a slightly compressible fluid. Three types of DPD particles have been used to represent the liquid, biofilm and substratum: they move according to a combination of conservative, dissipative, fluctuating and external forces. Their velocities and positions are calculated through the motion equation which is integrated by using a modified Verlet algorithm. Each DPD particle holds a specific mass of substrate and biomass which are individuated through a substrate concentration and a biomass density. Substrate dynamics for the biofilm and liquid DPD particles are governed by an advection-diffusion-reaction equation. The DPD representation of such equation includes the exchange of substrate among neighboring particles due to the concentration gradient and the thermally induced fluctuations. Biomass density varies according to a kinetic equation that incorporates biomass decay. When biomass density exceeds a maximum value, the excess is transferred to the nearest DPD fluid particle which in turn switches its status to a biofilm particle. In absence of fluid particles within the cutoff range, the biomass excess is assumed to be lost: this implies that the biomass mainly grows at

the interface between the biofilm and the fluid and it decays in the internal part of the biofilm. The model has been applied to the 2D case reproducing the biofilm growth in a narrow channel.

4 Other biofilm models

Although this review is mainly focused on the underlying principles adopted to describe the biofilm structure and spreading, we do wish to mention further models, that have been formulated on the basis of the above described biofilm growth models. They incorporate some specific ecological and biological aspects, such as QS, persisters dynamics, antibiotics and antimicrobials resistance and microbial interactions in multispecies biofilms. These applications are just some samples of the variety of biological questions requiring model extensions. They were selected relatively to their impact on biofilm dynamics and functionality. In the following subsections, we will provide essential information about these processes and the related models as the discussion of the various biofilm model applications is not being considered as the main scope of this work.

4.1 Quorum sensing

In the original paradigm, QS has been designated as one of the communication mechanisms that a huge number of bacteria use to monitor their own population density or to control the expression of specific genes after population density changes (von Bodman et al. 2008). This mechanism is based on the production and release of signaling molecules (autoinducers) into the surrounding environment, that can be sensed by the bacteria for intraspecies or interspecies communication. However, recent experimental findings have highlighted that many factors besides the cell density, such as spatial distribution of cells, diffusion and advection, pH and temperature, are involved in this mechanism (Kim et al. 2016; Pérez-Velázquez et al. 2016). A new paradigm which introduces the concept of efficiency sensing unifies most of the theories on this topic. It was proposed by Hense et al. (2007) based on theoretical considerations. The concept of efficiency sensing states that cells measure a combination of cell density, mass-transfer properties and spatial distribution to estimate the efficiency of producing extracellular effectors and react accordingly (Hense et al. 2007) for individual and community fitness benefits. Quorum sensing has been found to play a significant role in biofilm formation, maturation and dissolution (Hofer 2016; Li and Tian 2012; Solano et al. 2014) and a consistent amount of mathematical models, mostly in the last decade, has been developed to elucidate the basic mechanisms and their effects on biofilm growth and activity (for a survey see Klapper and Dockery 2010; Pérez-Velázquez et al. 2016). The first population-level models including quorum sensing were contextually developed by Ward et al. (2001) and Dockery and Keener (2001), who described this phenomenon in suspended bacteria. In their works, the switch from down-regulated to up-regulated sub-populations as positive feedback of quorum sensing systems was investigated (Frederick et al. 2011). The first deterministic continuous biofilm models incorporating QS, were presented/studied in 1D space (Chopp et al.

2002; Ward et al. 2003) by following the approach for the biomass transport mechanism introduced in (Wanner and Gujer 1986). These pioneer works were mainly devoted to the identification of the key physical parameters required for induction and were later extended to account for the effects of hydrodynamics and nutrient availability (Shrout et al. 2006; Chopp et al. 2003; Janakiraman et al. 2009; Vaughan et al. 2010) and anti-quorum sensing treatments (Anguige et al. 2005, 2006), which might represent a more efficient application than antibiotics agents in treating some biofilm infections (Hentzer and Givskov 2003). A 2D continuum model based on the approach of Eberl et al. (2001) was introduced in (Frederick et al. 2010) to investigate the contribution of flow to mass transfer and the inter-colony communication. The model focuses on the quorum sensing induction patterns in patchy biofilms, in a creeping flow regime, and distinguishes between the down and up-regulated bacterial sub-populations according to the paradigm introduced in (Müller et al. 2006). The model would be extended later to investigate the QS regulated EPS production (Frederick et al. 2011) and its well-posedness was proved in (Sonner et al. 2011). In (Emerenini et al. 2015), the authors extended the model of (Eberl et al. 2001) to study the QS induced biofilm detachment in a hydrostatic environment where the nutrients are transported to the biofilm from the aqueous phase by a diffusion gradient. The analysis of this model has been performed recently (Emerenini et al. 2017). In a recent work, Zhao and Wang (2017) extended the biofilm growth model developed in (Zhang et al. 2008a) to study the biofilm formation and development regulated by QS in an aqueous environment under hydrodynamical flows and possibly antimicrobial treatment. In (Ward and King 2012), the authors described the early biofilm growth and development and QS process by treating the biofilm as a viscous fluid and adopting a thin-film assumption. Following the biofilm simulation framework introduced in (Xavier et al. 2005b; Picioreanu et al. 2004a), an IbM was developed to investigate evolutionary competitions between strains that differ in their EPS production and QS phenotypes (Nadell et al. 2008). Fozard et al. (2012) modeled the inhibition of QS as a stochastic process on the level of individual cells by using the generic IbM described in Sect. 3.3.

4.2 Antibiotics and antimicrobials resistance

The biofilm growth mode induces microbial resistance to physical disruption and disinfection leading to substantial economic concerns, mostly related to health-care settings (Bridier et al. 2011; Stewart and Costerton 2001). Indeed, bacteria living in biofilms display much higher resistance to both antibiotics and biocides, by about 10–1000 fold, when compared to the free planktonic cultures (Zhang 2012). According to Bridier et al. (2011), such inherent hardiness and resistance is related to the heterogeneous and multifactorial biofilm structure and results from a combination of different mechanisms. The latter include physical protection of the bacteria by the surrounding extracellular matrix through the slow or incomplete penetration of the disinfectant and adsorption, physiological resistance arising from nutrient gradients formed by the bacterial spatial distribution within the biofilm and the existence of phenotypic variants characterized by slow growth, persistence and virulence as it will be specified in Sect. 4.3 (Cogan 2013).

The first biofilm models accounting for disinfection were formulated as 1D continuum models following the approach of Wanner and Gujer (1986) and Wanner and Reichert (1996) and addressed the following topics: physiological reduction in susceptibility or antibiotic consumption (Stewart 1994; Stewart et al. 1996; Sanderson and Stewart 1997), stoichiometric and catalytic transport of antimicrobial agent (Dodds et al. 2000), localized nutrient limitation and slow growth (Roberts and Stewart 2004), adaptive response (Szomolay et al. 2005, 2010). In the above mentioned works, the effects of the fluid dynamics on biofilm structure and biocide action were neglected and the bulk liquid was modeled as a well-mixed chemostat. In (Cogan et al. 2005), the role of fluid dynamics on the deployment of a biocide was investigated. The biofilm is supposed to be stationary even after disinfection. The velocity field is assumed to be relatively slow and Stokes equations are applied. Such equations are solved by using the method of regularized Stokeslets. A diffusion/advection equation is considered for the biocide including the chemical reaction between the biocide and the biofilm. The work introduced in (Cogan et al. 2005), was later extended to mainly account for the coupled motion of the biofilm and the liquid flow (Cogan 2008, 2010, 2011). In particular, in (Cogan 2008) the biofilm is treated as a viscous fluid, of higher viscosity than the external fluid, which is governed by Stokes equations like the biofilm. Biofilm growth is still neglected and thus both fluids are treated as incompressible. The model in (Cogan 2008) was further extended to account for fluid/biofilm motion and interactions in a thin channel, including the biomass and exopolymeric substance production, the persisters dynamics and the reaction between the biofilm and the antimicrobial agent (Cogan 2010). For the latter, a consumption term is introduced which is a function of the EPS concentration, as it is assumed that there is a stoichiometric reaction consuming both polymers and antimicrobial agents. A nonlinear, degenerate diffusion-reaction model formulated on the basis of (Eberl et al. 2001) and taking into account the two particulate substances active and inert biomass, was introduced in (Eberl and Efendiev 2003) to describe the diffusive resistance mechanism typical of biofilms. The disinfection rate is assumed to be proportional to the local disinfectant concentration and active biomass. The model was formulated for heterogeneous biofilm morphologies, but only applied to a 1D simulation study. In a further work (Efendiev et al. 2008), the authors focused on the disinfection process in spatially structured irregular biofilm morphologies, such as mushroom type cluster-and-channel architectures. The model has been studied both qualitatively and numerically for hydrostatic environments. It has been extended in (Eberl and Sudarsan 2008) to account for the convective contribution to the transport of dissolved substrates and disinfectant in the aqueous environment. A thin-film approximation to the Navier-Stokes equations has been considered to solve the fluid flow analytically. Reynolds number in the range of $10^7 \approx 10^2$ have been considered and the biofilm has been treated as a rigid nondeformable body. However, this simplification can be used only for slow flows in narrow channels. Moreover, the model neglects the biofilm alterations induced by the flow field. The model developed in (Zhang et al. 2008a) was applied to describe the biocide action against biofilm (Zhang 2012). The latter is treated as a mixture constituted by two viscous fluid components, the solvent where the biocide is dissolved and the biomaterial which in turns consists of live and dead cells. The whole system (solvent + biomaterial) is considered as an incompressible fluid whose aver-

age velocity is governed by Navier–Stokes equations (the creeping flow assumption is relaxed and Re numbers are kept in laminar regime for all the applications). Biofilm growth is neglected while the effect of the biocide on the mechanical properties of the biofilm is investigated. The model consists in a system of reaction/diffusion/advection equations governing the dynamics of the biocide, live and dead cells. The biocide is consumed only by active cells following Monod kinetics; the decay rate of the active bacterial cells due to the biocide action is also modeled through Monod kinetics and corresponds to the growth rate of dead cells. The intermixing between the solvent and the biofilm components is modeled by considering the variational derivative of the chemical free energy, as described in (Zhang et al. 2008a, b). The velocity field is evaluated through Navier–Stokes equations, which contain an additional term related to the forces arising from the chemical free energy of the mixture. In the modified Navier–Stokes equations, the viscosity of the whole system is expressed as a function of the biocide concentration, the untreated biomaterial and solvent viscosities and fractions. The dependence of the viscosity on the biocide is introduced in order to take into account the effect that the biocide exerts on the mechanical properties of the biofilm. In particular, the viscosity of the mixture is assumed depending on the biocide history as its application liquefies the biofilm and thus reduces the mixture viscosity. The first example of a stochastic model incorporating the action of an antimicrobial agent was introduced in (Hunt et al. 2005), where the spatial movement of bacterial biomass is described by the cellular automaton developed in (Hunt et al. 2003). The antimicrobial efficacy is simulated to be proportional to the amount of available substrate in order to simulate the behavior of a substrate-dependent antimicrobial. The model was further extended to test four hypothetical mechanisms for biofilm protection against antimicrobials (Chambless et al. 2006).

4.3 Persistence

During the last years, the phenomenon of persistence has gained an increased attention in the context of the biofilm as the latter provides an appropriate environment for the growth of slow-growing or non-growing bacteria (Balaban et al. 2013; Helaine and Kugelberg 2014; Lewis 2007, 2010). Some time-dependent, spatially homogeneous models on the possible mechanisms of persister formation have been introduced: most of them are essentially based on the classification of the bacterial population in two sub-groups: the persisters and the susceptible individuals (Cogan 2006, 2007b; Cogan et al. 2012). The persisters do not reproduce considerably while the second ones reproduce and are killed at a rate proportional to the growth rate (Cogan 2013). Roberts and Stewart (2005) investigated through a Wanner–Gujer biofilm model whether persister cell formation could confer increased protection from antimicrobial agents to a population of biofilm microorganisms. Persisters have been supposed to be generated at a fixed rate, independently of the presence of substrate or antimicrobial agent, and incapable of growth.

In (Cogan et al. 2013), current hypotheses on persisters were used to develop a 1D biofilm model based on the approach of Wanner and Gujer (1986). More precisely, persisters can reverse to susceptible state at a slow rate which is inhibited by the pres-

ence of the antimicrobial agent. The susceptible individuals can convert to persisters at a rate that depends on the nutrient level. Disinfection rate depends on the growth rate. The study is aimed at highlighting the effect of persisters on the dynamics of biofilm disinfection and it is interesting to notice how it deals with a qualitative analysis of the biofilm dynamics in one-dimension, proving the existence and stability of the steady-state solutions. Moreover, the results of the numerical simulations provide relevant indications and observations about the interplay between the period and the optimal fraction of disinfection. A more complex model that combines the concept of adaptation and persistence, based on the modeling approaches in (Szomolay et al. 2005) and (Cogan et al. 2013), has been introduced recently (Szomolay and Cogan 2015). The model incorporates physical and physiological mechanisms and both adaptive stress response and persistence in 1D biofilm model. Based on the observations that bacterial cells can demonstrate aging effects, a different modeling approach was introduced in (Klapper et al. 2007) where the authors treat the persisters as senescent cells, which are slow-growing cells and show higher tolerance to antimicrobial agents. This new approach introduces a partial differential equation for the population density of senescent cells, which depends on time and age.

Lately, a multiphase hydrodynamic model for biofilms of multiple bacterial phenotypes, including persisters and susceptible bacteria, has been introduced in (Zhao et al. 2016a). The model extends the work of Zhang et al. (2008a) by distinguishing between the persister and susceptible cells when biofilms are treated by antimicrobial agents. The interplay among the various biomass components such as various bacterial types, EPS and solvent is carefully taken into account both hydrodynamically and chemically.

4.4 Microbial interactions

The majority of biofilms contains multiple bacterial species and in many cases also fungi, algae, and protozoa. Such complex communities are characterized by interspecific interactions that can be categorized as either competitive or cooperative. These can be direct (cell–cell interactions) or indirect (co-metabolism and syntrophy) (Burmølle et al. 2014; Liu et al. 2016). These communal interactions often lead to emergent properties in biofilms which have been shown to provide benefits to all biofilm members, such as enhanced tolerance against antibiotics, host immune responses and other stresses, and formation of environmental microniches where different bacterial species coexist and contribute to the treatment of various compounds (Boltz et al. 2017). Specific direct interactions, also known as co-aggregation, are well described for oral biofilms (Marsh and Zaura 2017). Co-metabolism interactions are the foundation of many environmental remediation technologies, and the most striking examples can be found in wastewater treatment (Muhammad and Eberl 2011). A large variety of mathematical models have been developed to assess the multispecies population dynamics in different biofilm systems applied to wastewater treatment. Traditional Wanner–Gujer based models implemented through the well-established AQUASIM simulation software (Reichert 1994) have been widely used to both understand and ultimately control the microenvironment in terms of optimal community structure and spatial organization in a wide range of applications (see Wolf et al. 2007; Lackner et al. 2008; Wang

et al. 2009; Volcke et al. 2012; Vannecke et al. 2016 as examples). More complex 2D and 3D models, either continuum or discrete, have been developed to represent the spatial heterogeneities of bacterial species establishing in vertical as well as horizontal directions (Hauser and Vafai 2013). A 2D continuum model based on the framework of (Alpkvist and Klapper 2007) and capable of handling multiple species and multiple diffusive components in two space dimensions was developed to describe a nitrifying moving bed biofilm reactor and applied to a test facility of a municipal wastewater treatment plant (Alpkvist et al. 2007). This framework is coupled to a global mass balance model at the reactor scale. A multispecies multicomponent biofilm model (Merkey et al. 2009) was developed combining the approaches in (Laspidou and Rittmann 2002b; Alpkvist and Klapper 2007) to evaluate the dependence of multispecies coexistence on soluble microbial products and EPS in a heterotrophic/autotrophic biofilm. The mentioned model has found recent application in the simulation of microbial fuel cells (Merkey and Chopp 2012, 2014). The work in (Batstone et al. 2006) represents one of the first applications of the IbM approach (Picioreanu et al. 2004a) to model the syntrophic interactions establishing between fermentative bacteria and methanogens in anaerobic granules. The model domain is limited to a single granule, with fixed bulk liquid concentrations. In (Xavier et al. 2007), a 2D multiscale model for an aerobic granular sludge sequencing batch reactor was introduced to understand the effect of operating conditions on the composition of the microbial population, as well as the reactor performance in terms of nitrification, denitrification, and phosphorus removal efficiency. The model makes use of an IbM framework (Xavier et al. 2005b) for the metabolism of individual biomass elements (individual scale) and the spatial structure of the microbial population in the granule (granule scale), which will be first integrated with the reactor scale sequencing batch dynamics (macro scale). Four bacterial groups will be considered: ammonia oxidizing bacteria, nitrite oxidizing bacteria, nonphosphate accumulating heterotrophs, and phosphate accumulating organisms. The model has been further applied to the case of a nitrifying granular reactor fed with ammonia as the sole energy source (Matsumoto et al. 2010) or to describe the start-up of an aerobic granular sludge system (Kagawa et al. 2015). A 3D dual-morphotype species model of activated sludge flocs was introduced in Martins et al. (2004), where the authors adapted the IbM previously developed (Picioreanu et al. 2004a) to simulate the 3D formation of activated sludge flocs. Two different bacterial morphologies, the floc-forming and filamentous bacteria, have been considered. Multidimensional models have also been applied to the case of emerging wastewater treatment technologies, such as membrane biofilm reactors and microbial fuel cells. A first attempt was introduced in (Matsumoto et al. 2007), where the authors on the basis of (Alpkvist et al. 2006) developed a 2D hybrid computational model to describe the simultaneous nitrification/denitrification process and simulate the micro-environment in an oxygen-based membrane reactor. As to simplicity, the fluid flow and the shear patterns have not been considered the biofilm being assumed to grow on a flat surface. A 2D multispecies biofilm model was developed in Martin et al. (2015) to study the microbial competition in a hydrogen-based, denitrifying membrane reactor. The effect of detachment on microbial interactions in a biofilm with a non-flat attachment surface was investigated. The biofilm sub-module is implemented according to Picioreanu et al. (2004a) while the hydrodynamics, mass transport by diffusion and

advection are treated as in (Martin et al. 2013). Various biofilm models have also been developed to elucidate the microbial activity in microbial fuel cells, as it represents one of the most effective factors regarding this technology (Ortiz-Martínez et al. 2015). The selection of electroactive bacteria in the anodic compartment was investigated in (Picioreanu et al. 2007b). In particular, it was assumed that acetate can feed two competing microbial populations independently: electroactive bacteria and methanogenic bacteria. The biofilm sub-domain is modeled following (Picioreanu et al. 2004a; Xavier et al. 2005b). The dynamics of the microbial community (electroactive biofilms with methanogenic and fermentative microorganisms) as well as the effect of pH and electrode geometry were further investigated in (Picioreanu et al. 2008) and (Picioreanu et al. 2010) respectively. Starting from (Tang and Valocchi 2013), a 2D cellular automata model has been developed recently to investigate the population dynamics for the in-situ bioremediation of uranium contaminated groundwater (Tang and Liu 2017). The model considers multiple interactions among fluid flow, transport and reaction of chemical species, and the growth of two types of active biomass (syntrophs and dissimilatory metal reducing bacteria) and inert biomass. The two types of active biomass collaboratively remove uranium.

5 Discussion

Due to biofilm involvement in a large range of human activities and natural processes, developing an effective mathematical modelling approach may be essential to elucidate the processes involved in biofilm formation and maturation, as well as to implement a strategy to minimize the biofilm related risks and exploit their technical possibilities. The heterogeneity of the biofilm structure and the interdependence of physical/chemical/biological processes, occurring at different time and space scales, make mathematical modeling of biofilm growth and structure a best challenge for researchers.

A detailed overview of the wide range of modeling approaches developed during the last decades, was presented above. Selecting an appropriate model may represent a challenging issue for both researchers and practitioners (Morgenroth et al. 2000). The scope and output of the model constitute a discriminator factor: practitioners are interested in developing models able to quantitatively predict the performance and responses of biofilm reactors; researchers consider modeling as a powerful tool to understand the fundamental mechanisms regulating the formation and performance of biofilms. Therefore, practitioners aim at working with simpler biofilm models, which can be easily calibrated by using the data provided by experimental activity. In research though, the degree of a model complexity has been increasing in the past few years.

On the basis of the model classification proposed in this work, we are going to provide general guidelines for the selection of the most suited modeling tool, related to the specific needs of the model user. In particular, two general questions are answered in the following sections, which should ban any doubts arising in choosing a modeling approach: i) When it is best to use 1D, 2D or 3D models? ii) Which approach should be used? Continuum or discrete? However, there is no general answer to these questions because the choice of a specific modeling tool is objective depending. For instance,

different environmental conditions produce a variety of biofilms, which are structurally very different one from the other. Some mathematical models are able to capture this heterogeneity; others, based on simplifying assumptions, are not. The extent to which simplifications and idealizations must or can be introduced depends on the particular purpose of the mathematical model (Eberl 2003).

5.1 When it is best to use 1D, 2D or 3D models?

As described in details above, 1D models consider only the direction perpendicular to the substratum: this represents a valid simplification when vertical gradients of variables and parameters are orders of magnitude higher than those in the directions parallel to the carrier surface (Xavier et al. 2005a). This hypothesis verifies in the case of uniform bulk liquid conditions over the whole substratum area, when the substratum area is regular and rather large if we compare it to biofilm thickness or in case of smooth biofilm surfaces. As those assumptions apply to many (not all) engineering biofilm systems, 1D models have been widely used to predict the whole process dynamics of biofilm reactors and are increasingly used as educational material in engineering curricula (Boltz et al. 2010). More precisely, the choice of 1D models rather than a more complex multidimensional formulation is mainly oriented by the type of outputs the user is interested in. Biofilm model outputs can be classified on the length scale in macro-scale and micro-scale outputs. The former includes the overall substrate flux, the degradation rates, the external mass transfer limitations, the biomass accumulation in the biofilm and the biomass loss from the system (Wanner et al. 2006). Conversely, micro-scale outputs include the spatial distribution of substrate and microbial species within the biofilm (not only in the direction perpendicular to the substratum), physical structure of the biofilm at the μm-scale, shape and local density of the biofilm solid matrix. If the user is interested in macro-scale outputs, as the case of engineering applications of biofilm reactors, 1D models have been recognized as valuable and efficient tools to analyze the reactor performance and to provide reliable information about the mass of the microbial species, substrate removal rates and effluent concentrations (Wanner et al. 2006). However, for this type of models the user should be aware that the typical biofilm features, such as cluster segregation, cannot be taken into account. In addition, the choice of 1D models reflects the need of keeping the computational effort at a low level. An inherent limitation of 1D models relies on the simplified modeling of bulk liquid as a completely mixed compartment. The calculations regarding the flow field are neglected as well as the interactions between liquid flow and biofilm surface.

Recent improvements in microscopy and imaging techniques have revealed that numerous biofilms are not uniform. In fact, in real biofilms spatial irregularities cannot be interpreted using a conceptual model of biofilms where microorganisms are uniformly distributed in a continuous matrix of extracellular polymers (Beyenal and Lewandowski 2005). Biofilms have been recognized as complex 3D heterogeneous entities characterized by a highly porous structure filled with fluid, which supplies microorganisms with nutrients and erodes the biofilm surface leading to the biomass removal. This spatially heterogeneous architecture can induce complex flow patterns and affect the mass transfer (Wanner et al. 2006). 1D models result inappropriate to

describe the dynamics of biofilm activity when structural dependent factors such as external mass transfer coefficient or porosity significantly vary with time. Therefore, 2D and 3D models were developed to capture this heterogeneity. Multidimensional models are able to evaluate the substrate removal and biomass production rates of dynamic biofilm systems (macro-scale outputs), but they can be also used to evaluate the interaction among the biofilm shape, the fluid flow, the biomass decay and detachment. This is accomplished by taking into account the fluid dynamics modeling of the liquid phase and the effect of biofilm geometry on the external mass transfer rates. Those models are suitable to study the effect of different environmental conditions on biofilm structure and to evaluate the multidimensional interactions between microbial communities, such as microbial segregation (micro-scale outputs). 2D models are not representative of a 3D domain where flow is able to by-pass dense biofilm structures (Wanner et al. 2006). However, simulations performed by Eberl et al. (2000) highlighted that in most cases, 2D and 3D models lead to equivalent external mass transfer coefficient. On the other hand, 3D models can be useful for biofilms consisting in isolated colonies where advective transport becomes not negligible. The development of highly accurate 2D and 3D models requires a detailed description of the biofilm structure at a meso-scale, through the use of modern investigation techniques, together with the solution of nonlinear systems of partial differential equations in a complex domain. In addition, multidimensional biofilm models have been singularly used as research tools, where an accurate resolution of the inside processes is required. Their application as engineering tools is limited by the high spatial resolution and the detailed level required for model calibration.

Based on the results achieved in the Benchmark problems discussed in (Wanner et al. 2006), the following considerations can be drawn on the use of 1D versus multidimensional models. When applied to the case of simple single-species biofilm systems, the bulk liquid being assumed as a completely mixed compartment, 1D models are able to provide modeling results (i.e. substrate and oxygen fluxes across the biofilm interface and their concentrations in the bulk liquid, at the biofilm surface, and at the substratum) in agreement with the more complex multidimensional formulations. The latter reproduce a heterogeneous structure that is characterized by an average thickness equal to the one attained by 1D models. However, simulation results have revealed some inherent modeling issues that should be taken into account: different mass transfer assumptions for rough biofilm surfaces have a major influence on predicted substrate bulk liquid concentrations and fluxes. Besides, biofilm morphologies strongly affect the modeling outputs when small bulk liquid concentration and penetration depth of the limiting substrate are obtained (Wanner et al. 2006). In a recent work, it has been argued that for large scale physical phenomena, substrate transport homogenization is applicable when the heterogeneity scale is small compared to the active layer depth (Aristotelous et al. 2015). When the hypothesis of a completely mixed bulk liquid is dropped and fluid field calculations are required, the Benchmark problem gets addressed to analyze to which extent the application of 2D or 3D modeling approaches is necessary to elucidate macro-scale biofilm behaviors. The comparison among simulations of 3D and 2D selected models highlights that globally the dimensional reduction of hydrodynamics calculations results in a more significant aspect than geometrical description.

5.2 Which approach should be used? Continuum or discrete?

Continuum models represent a valid alternative to the discrete approach since all the drawbacks that characterize discrete models seem to arise from the discreteness of the adopted spreading mechanism (Eberl et al. 2001). As already mentioned, a continuum biofilm model: (i) is characterized by a continuous representation of biomass; (ii) is based on differential equations widely used in physics to model the dynamics of biomass spreading and (iii) generates deterministic solutions. The main advantages of continuum biofilm descriptions derive from the use of the powerful framework of partial differential equations. Indeed, the use of differential calculus allows the achievement of quantitative results for the substrate transport that can be compared with data measured in real systems. Today, Tthe 1D continuum models are widely used methods to describe macroscopic conversions and to interpret and predict the biofilm reactors performance (Xavier et al. 2004a). The 1D dynamic multispecies model of Wanner and Reichert (1996) implemented in the software AQUASIM, is up to date the most widely used biofilm model applied to engineering design, as it is sufficiently accurate for predicting the global mass conversion rates for a full bioreactor. However, 1D models do not provide any information about the local spatial architecture of the biofilm. Multidimensional continuum models are being developed to cover this gap. The main challenges in developing multidimensional continuum models rely on the presence of moving boundaries, i.e. the biofilm-fluid interface, fluid flow, non-linear growth kinetics and discontinuous gradients across the boundary biofilm-fluid interface (Duddu et al. 2008). In particular, the use of multidimensional continuum models implies high computational efforts and sometimes it requires simplifying assumptions to solve the differential equations describing the biofilm evolution on irregular domains. Flow field calculations are usually much more computationally expensive than simulations of biofilm growth. Therefore, bulk flow hydrodynamics has usually been neglected in many multidimensional continuum models. Moreover, a variety of resolution methods has been investigated, being characterized by significant computational efforts to solve elliptic equations on irregularly shaped domains in conjunction with moving interfaces. The formulation and derivation of continuum models require a comprehensive mathematical skill, a higher computational effort compared to discrete methods and at times not trivial computational algorithms (Alpkvist et al. 2006). Despite the high computational efforts, multidimensional continuum models are more convenient than discrete approaches when applied to mechanical problems, which necessarily imply flow field calculations. Indeed, in discrete models the flow field has to be recomputed with every realization of the stochastic process simulated, which presumably makes these models inefficient (Sudarsan et al. 2015). Moreover, in the optic of computational tractability, discrete models make use of a simplified approach to reproduce mechanical deformation and biofilm physical properties which are, in turn, dominated by the slime matrix rather than the cellular component. This simplified view might affect the surface architecture which strongly depends on the way the stresses that are induced by the growth are accommodated (Fowler et al. 2016a).

On the other hand, discrete models are able to represent the typical multidimensional structural heterogeneity of biofilm in good agreement with experimental expectations, but they generate computational results that include elements of randomness. Their

outputs depend on the sequence of execution of methods on the discrete objects and introduce stochastic effects into the solutions. In the case of IbMs, the stochasticity manifests itself in two occasions: (a) in the random choice of direction for the placement of "daughter" particles and (b) in the uneven mass division between cells (Xavier et al. 2004a). For CA, the rules used for biomass spreading are sometimes arbitrarily formulated and might lead to aesthetically driven, rather than to physically motivated, model formulation (Eberl et al. 2001). Generally speaking, they are lattice dependent and not invariant to changes of coordinate system. Moreover the same initial conditions can lead to different model outputs and error analyses are non-trivial (Alpkvist et al. 2006). Therefore, for a discrete model, before getting to any conclusions several runs with the same initial state are needed to average the stochastic effect. Despite the intrinsic stochasticity which characterizes the outputs of this type of models, no statistical analysis of the simulation results is usually carried out, making model fitting more cumbersome. In addition, although the modeler can directly control the entity of the mixing generating from the merging of colonies of different species through specific local interactions rules, CA models have been often criticized for overemphasizing the mixing. That aspect becomes crucial when the main scope of the model is to elucidate the local effects within biofilm colonies in terms of cell distribution (Sudarsan et al. 2015). To minimize the excess of mixing, several types of spreading rules were adopted over the years (Laspidou and Rittmann 2004b; Noguera et al. 1999a; Tang and Valocchi 2013). That criticism is also faced by a class of continuum models (Eberl et al. 2001) which overpredicts mixing and reduces biomass gradients due to the model's pure self-diffusion character. A further drawback arises from the use of the same lattice grid for the discretization scheme adopted to solve the substrate diffusion equation and the biomass CA lattice, as most authors skip to show whether this resolution is fine enough or it leads to mass balance closure. Despite the aforementioned disadvantages, both discrete approaches, CA and IbM represent powerful modeling tools which have been applied not only in ecology, but in many other disciplines such as social, economical, demo-graphical and political sciences. Similarly to multidimensional continuum models, CA work on a larger scale than IbMs, which are usually used for studies at the scale of micrometers to centimeters and therefore are computationally more intensive. According to Laspidou et al. (2010), CA are especially suitable to model old and aged biofilms, characterized by the presence of cavities and experiencing the phenomenon of consolidation.

In IbMs, the collective action of each individual determines population or community level properties and the feedbacks between the behaviour of individuals and the population as a whole, emerge automatically (Hellweger et al. 2016). These models are mainly addressed to capture the micro-scale level and to describe how individual processes, interactions and local variability affect the macroscopic structure of biofilms. One of their main drawbacks, relies on the assumption of individual microorganisms as hard spheres and on the use of a predetermined shoving parameter to model the direction of the biomass movement and porosity. Furthermore, information in individual heterogeneity of growth parameters, the volume fraction occupied by cells in colonies and the biomass spreading mechanism adopted by different microorganisms are sometimes missed. Limitations of the IbMs include the feasibility of modeling large-scale systems, the availability of individual-data and the tendency to become

too complex and difficult to be mathematically analyzed. In the case of large-scale systems the use of IbMs would become necessary only when the modeling of the intracellular mechanisms giving rise to heterogeneity is desired. Indeed, stochastic differential equation models which make use of continuous probability distribution and are independent from the scale of the system, could be used on their place. The lack of specific individual-based data related to single cell observations makes IbMs mere tools to test hypotheses on the distribution of single-cells properties (Hellweger et al. 2016). Despite their computational demand, the IbM approach can incorporate rare species or events, it can make a distinction between spreading mechanisms adopted by different bacteria and operates at the highest spatial resolution level relevant in a biofilm (Picioreanu and Loosdrecht 2003). IbMs represent a big promise in modelling multispecies biofilms and in incorporating concepts such as cell-to-cell signaling, quorum sensing and cellular motility.

The intrinsic features of the continuum and discrete models induce to consider them like opposite approaches. However, it is worth remarking the existence of a mathematical relationship between the discrete and continuous models. That link has been highlighted in (Rahman et al. 2015; Khassehkhan et al. 2009b) where the authors derived a degenerate diffusion model for a single-species biofilm growth starting from a discrete master equation typically used in theoretical ecology as well as to describe cell movement such as chemotaxis. Such master equation reproduces the probability that bacteria move from one site on a regular lattice into a neighboring site (and vice versa) resembling the CA algorithm. However, the transfer of biomass among lattice cells is not defined by means of discrete stochastic rules, but the mass transfer rates are described as continuous monotonously increasing or decreasing functions. The master equation also accounts for the net biomass production rate in each cell grid. The so obtained semi-discrete master equation model applies to the derivation of the deterministic continuous model by refining the spatial discretization and passing to the continuous limit.

6 Conclusions and future directions

Biofilm development and growth involve many processes, including cell-surface and cell-cell adhesion, biomass spreading, substrate diffusion, fluid flow interactions and biofilm matrix rheology (Nadell et al. 2016). Biofilm models have been recognized as useful tools for studying and exploring such fundamental processes on a wide range of temporal (from nanoseconds to hours or days) and spatial (from nanometer to millimeter length) scales as well as elucidating microbial competition and coexistence. The main goal of this review is to present an up-to-date landscape of the modeling approaches proposed to describe biofilm development and structure, with particular regard to biomass representation and spreading (Mattei 2014). We have shown that the models are characterized by several useful features and numerous differences, they can be classified in two broad categories, namely the continuum and discrete models. Continuum models benefit from the framework of differential calculus and represent a valuable tool to understand the biofilm processes in a quantitative and deterministic way. However, 1D continuum models assume a planar geometry and therefore, they

cannot take into account the biofilm spatial heterogeneity. These early models have been proposed to aid in the design and maintenance of various industrial reactors as well as wastewater plants. They are still used in a variety of industrial settings where the desired outputs, including removal rates and effluent concentration of dissolved components, mainly rely on the dynamics of the biofilm intended as a whole system (Cogan et al. 2011). On the other hand, the formulation of multidimensional continuum models, which started to be developed with the aim of elucidating the emerged structural and physical complexity of biofilms through the use of differential calculus, requires comprehensive mathematical skills and sometimes, challenging and demanding numerical techniques. Discrete models are more recent in time in the biofilm research and they are based on the idea that biofilms can be characterized like stochastic living systems. These models have shown their capability of representing the biofilm structure heterogeneity in good agreement with experimental results. However, they introduce elements of randomness, mostly in modeling the spreading of biomass as they lead to structured shapes resembling biofilms but they may not simulate the exact reality. Nevertheless, despite the impressive advances in our biological and modeling knowledge of biofilms, we remark that the use of biofilm models to elucidate and reveal new features and behaviors of such complex communities is far from being completely explored (Cogan et al. 2016). Although there has been much effort in the incorporation of the interplay between biofilm growth, mechanics and hydrodynamics, the quantitative modeling of the relationship among the motion of the external fluid, deformation, growth and detachment of immersed biofilms with the employ of measured biofilm mechanical properties, remains one of the core concepts of the biofilm research, due to the high complexity and bi-direction of biofilm mechanics. Some modeling insights on the description of a biofilm growing in a fluid motion environment have been achieved by using continuum representations (Head 2016). However, most of them introduce a specific challenge related to the position of the biofilm/liquid interface which has to be tracked by using stress and displacement matching, which becomes non-trivial even with computational solution when complex, dynamic geometries are involved (Head 2016). Despite some models incorporate detachment to elucidate erosion and sloughing of the biofilm surface, not all of them take into account the two-way coupling between biofilm mechanics and fluid shear stress and thus do not accurately reproduce the biofilm structural and mechanical properties. Some insights on this crucial point could come from further developments on phase fields models or on the immersed boundary approach. The latter requires extension to include growth, dispersed-phase advection (Head 2016) and improvements in terms of adopted numerical strategy, as its full potential could be exploited by using an implicit time-stepping approach or an efficient parallel implementation (Sudarsan et al. 2015). A key research question that demands better investigation is related to the role that EPS composition exerts on the establishment of a specific biofilm structure. EPS has been found highly variable among microorganisms and even within a single species. A more accurate modeling description (microscopically or mesoscopically) incorporating its composition and interaction with the substratum and the bacterial cells themselves as well as its mechanical properties, is still missing. Such a model could help understanding biofilm development as EPS represents one of the main discriminators between the sessile and planktonic microbial lifestyle. A second aspect

that has been omitted in most of the developed biofilm models is motility, which plays a crucial role in the initial phase of biofilm development as well as in the last stages of the idealistic biofilm life-cycle via dispersal of individual cells. The latter has been recognized as a potential way to control and remove biofilms of industrial and clinical concerns (Cogan et al. 2016). Other research areas, that need further investigation, are related to intracellular interactions and communications, genetic changes/mutations and transfers, mechanical interactions or adaptive behavior in biofilms. For all the biofilm models, validation should be improved: this point demands a better integration of experiments and models and is needed to achieve a significant progress in our understanding. We conclude remarking that only the collaboration among researchers with different expertise will lead to the definition and development of a modeling approach (or a conjunction of existing modeling approaches), able to take into account the advances in biofilm ecology.

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